Journal of
DAIRY SCIENCE

Vol. 44 November, 1961 Number 11

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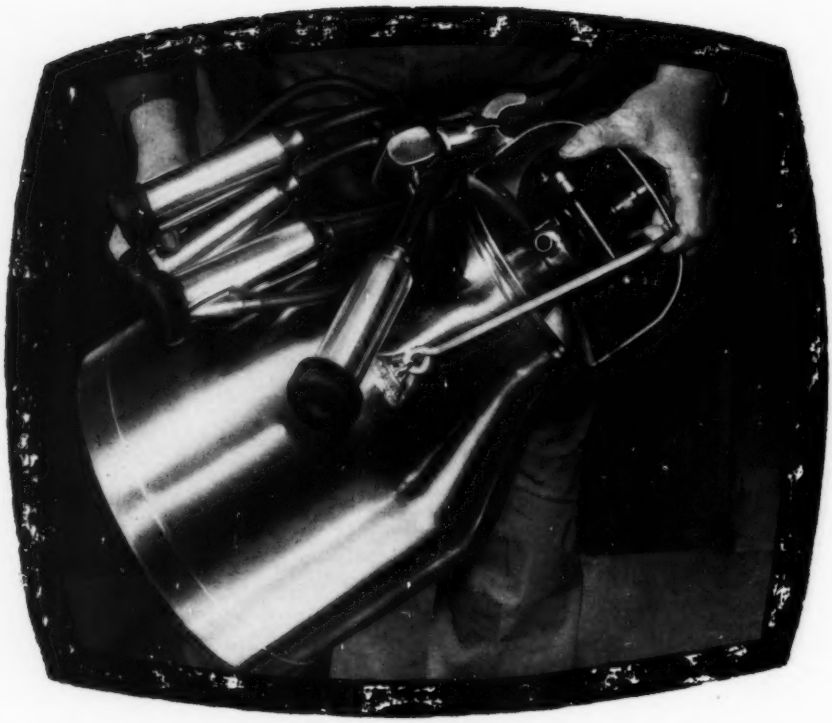

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B-K Powder with cleanser	0	6 mg.
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PEOPLE AND EVENTS

UNIVERSITY NEWS

Dr. Blosser Made Chairman at Washington

DR. T. H. BLOSSER, Washington State University dairy professor, has been named chairman of the dairy science department at that institution.

T. H. Blosser

Dr. Blosser has been a member of the WSU faculty since 1948. His research work has been in the fields of animal diseases and nutrition. He has done specific research projects in milk fever and the nutritional value of forage. He has supervised the work of 15 graduate students and is the author of more than 60 scientific and popular publications.

He is on the official judges' list for the Holstein and Guernsey breeds and has judged at dairy shows in Montana, Oregon, Utah, California, and Washington.

The new dairy science department chairman is a native of Indiana. He received his Bachelor's degree from Purdue University and his Master's and Doctor's degrees at the University of Wisconsin.

Ohio News

DR. T. KRISTOFFERSEN, Associate Professor of Dairy Technology, The Ohio State University, served as official judge of dairy products for the Michigan State Fair September 1, thereby reciprocating the services of PROFESSOR J. M. JENSEN, Michigan State University, who served in a similar capacity at the Ohio State Fair in late August.

One hundred Ohio State University College of Agriculture freshmen attended the Dairy Technology Exploratory Meeting held October 10. A program designed to inform students of the scope of Dairy Technology and the opportunities it affords was presented by faculty members and senior students. A tour of Department facilities, combined with discussions of Departmental activities, also was featured. The program was part of a broad College of Agriculture effort to provide students who are undecided as to careers with information about areas of endeavor represented by each of the college departments, as a means of facilitating their selection of a major field.

DR. R. E. EDWARDS, of Edinburgh, Scotland, visited the Department of Dairy Technology at Ohio State University during the week of October 2-6. Dr. Edwards discussed the Department's research program and specific aspects of the Ohio Dairy Industry with staff members. as part of an extended visit to this country to become familiar with the American Dairy Industry. While in Columbus, Dr. Edwards also met with research personnel at M & R Dietetic Laboratories.

The Annual Dairy Technology Bean Feed was held in Ohio State University's Vivian Hall the evening of October 3. Sponsored by the Ohio State Dairy Tech Club, this event is designed to provide an opportunity for entering freshmen to become further acquainted with their classmates, department staff, and the program of the club. Prior to the informal program, a touch football game between undergraduates and a graduate student-faculty team whetted appetites for the meal of beans, frankfurters, and dairy products. The program was highlighted by a presentation by KEITH JENKINS, vice-president of the OSU Dairy Tech Club, and second vice-president of the Student Affiliate Branch of the A.D.S.A., who reported on his representation of the club at the 1961 A.D.S.A. Meetings. JOHN MURRAY, Club president, spoke on club activities.

Connecticut
University Holds Career Day

A very successful Dairy Manufacturing Career Day was held at the University of Connecticut, October 28. Members of the Connecticut Association of Dairy and Food Sanitarians, under the chairmanship of E. I. KELLARSON, H. P. Hood and Sons, Hartford, Connecticut, recruited and transported the prospective students who attended from schools throughout the state.

The need for college-trained personnel and the opportunities in the dairy industry were emphasized by dairy industry speakers. Industry representatives appearing on the program included: J. J. EGAN, laboratory director, Connecticut Milk Producers' Association, Hartford; H. M. EWELL, sales representative, Pennsylvania Salt Manufacturing Company, Arlington, Massachusetts; DR. R. M. PARRY, Chief of the Dairy Division, State Department of Agriculture, Hartford; and GEORGE FARNUM, President of Connecticut Milk for Health, Hartford.

The day included a luncheon through the courtesy of the Connecticut Association of Dairy and Food Sanitarians and the Connecticut Milk Dealers' Association, a tour of the

dairy manufacturing facilities, and attendance at the Connecticut-Boston University football game.

North Carolina Faculty Changes and News

DR. GEORGE HYATT, JR., former Head of the Animal Industry Department, assumed his new duties as Associate Director of the Agricultural Extension at North Carolina State College, September 1. He will serve in this capacity for two years, at which time he will become Director. A member of the college faculty for the past nine years, he also served as Extension Dairy Specialist and chairman of the Dairy Extension Section prior to becoming Head of the Animal Industry Departmen.

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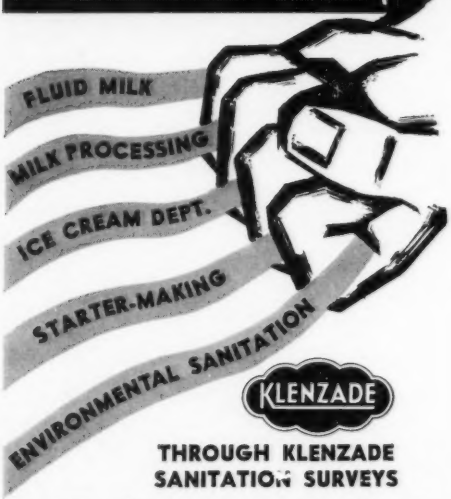

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T. C. BLALOCK, Head of the Dairy Extension Section, is taking a year's leave of absence to study toward his doctorate at the University of Wisconsin. J. D. GEORGE is Acting Head of the section in the absence of Blalock. W. E. MANIOUS, former Assistant County Agricultural Agent in Winston-Salem, is filling in as Extension Dairy Specialist while doing graduate work.

A surprise dinner was recently given for W. RAY MURLEY, Professor in Animal Industry, by former members of the dairy cattle judging teams he has coached during the last ten years. Twenty-five judging team alumni turned out for the occasion and presented Dr. Murley with a watch as an expression of gratitude for having gained so much while a member of the team.

Bids were recently opened on a contract to build a million-dollar addition to Polk Hall in which the Animal Industry Department is located. This new facility, when completed, will give staff members more than double the laboratory and office space now being used. Completion of the building is expected in 1962.

DR. J. M. LEATHERWOOD recently rejoined the staff of the Dairy Husbandry Section after spending a year on a post-doctoral fellowship at Duke University Medical Center in the Department of Biochemistry. He will continue to work in the area of cellulase enzymes and rumen function, in addition to some teaching responsibilities.

Dairy Industry Conference at Arizona

The Department of Dairy Science at the University of Arizona will sponsor the Eleventh Annual Dairy Industry Conference, January 17 and 18, 1962. It is expected that about 200 people will attend the meeting. In attendance will be dairy plant owners, managers, and operators; dairy equipment and supply representatives; and public health and other regulatory officials.

Guest speakers will include DR. I. A. GOULD, Head, Department of Dairy Technology, The Ohio State University; DR. J. J. SHEURING, Professor of Dairying, University of Georgia; and DR. G. P. Whitlock, Program Leader, Family and Consumer Services, Agricultural Extension Service, University of California. The speakers will cover subjects dealing with current problems and research in dairy plant operation and quality control.

Kentucky Scholarships Awarded

Scholarships were awarded six students in the Department of Dairy Science for the fall term at the University of Kentucky. Amounts varied from $100 to $150 per semester, depending on classification of the students.

DR. D. M. SEATH, Head of the Department of Dairy Science, made the awards in the name of the Kentucky Dairy Educational Committee. This committee is made up of dairy leaders representing dairy production and dairy manufacturing interests of the state. The group was formed to advise with and to support the educational efforts of the Department at the University of Kentucky.

Scholarships were awarded six students in Dairying by the Kentucky Dairy Educational Committee. Those receiving the scholarships are from left to right: Allen Chiles, J. W. Davenport, Jr., B. L. Hornback, B. J. Deaton, J. M. Peters, and T. D. Bishop.

The scholarships are awarded students on the basis of need and scholastic ability. A student must be a major in dairy production or dairy manufacturing and must maintain at least a 2.25 (on a 4.0 system) average. Second-semester freshmen and sophomores are awarded $100 grants per semester and juniors and seniors, $150.

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Those receiving the awards for the current semester and the amounts were: T. D. BISHOP, $150; R. A. CHILES, $100; J. W. DAVENPORT, JR., $100; B. J. DEATON, $100; B. L. HORNBACK, $150; and J. M. PETERS, $150. Hornback, Deaton, and Davenport are president, treasurer, and annual editor, respectively, of the University of Kentucky Dairy Science Club. The others are members of the club and are active on a number of committees.

New Buildings at Auburn, Alabama

Six new buildings at Auburn University, Auburn, Alabama, equipped with modern agricultural and veterinary sciences facilities, were dedicated October 6 to the advancement of Alabama agriculture through improved teaching and research.

The modern new facilities include: Animal Sciences Building, Funchess Hall (biological science), Lambert Meats Laboratory and Judging Arena, McAdory Hall (large animal clinic), and Sugg Laboratory (animal disease research).

The Sciences Building houses the departments of Animal Science, Dairy Science, and Poultry Science. The building includes the renovated original animal husbandry and dairy building constructed in 1928 and the recently completed new wing.

Animal Science Wing

Areas of research in the dairy science department are: evaluation of pasture, feed concentrates, and harvested forages; dairy chemistry, including studies on milk flavors, composition of dairy products, and other problems in dairy manufacture; and dairy bacteriology, including drug residues in milk following medical treatment of cows.

Together with the new teaching and research facilities, this department has launched a far-reaching program in fundamental research and instruction at the undergraduate and graduate levels in keeping with the rapidly growing animal industries in Alabama.

Illinois Top Team in Dairy Products Judging Contest

The dairy products judging team from the University of Illinois—two men and a girl—were victorious in the annual Collegiate Students' International Contest in Judging Dairy Products in Washington, D.C., October 23-24. For being the best all-round judges of dairy

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products, they won the All Products Bowl and the accompanying cash fellowship of $2,300.

Team star was D. T. GORDON, Des Plaines, Ill., who, in individual awards, won the gold watch for ice cream judging and silver medals for all products and cheese judging. J. C. ALWES, Bloomington, Ill., another member of the Illinois team, took the bronze medal for butter judging. The third member of the Illinois team was the only girl in the contest—Patricia Carter, Havana, Ill., who kept the team average up by being fourth high individual on all products. The team was coached by DR. JOSEPH TOBIAS.

INDUSTRY NEWS

Changes at Foremost Dairies

Foremost Dairies, Inc. has announced that F. H. MCDOUGALL has been named branch manager at Fortuna, California; and J. W. SEAMAS branch manager in San Rafael.

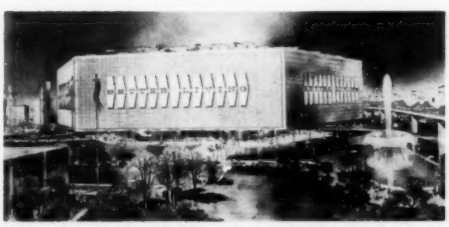
At the San Francisco headquarters of the company W. R. ROWE has been selected as general credit manager; D. G. CORBETT, JR., assistant product manager for packaged foods; and W. L. PALMER, JR. has joined the market research staff.

Foremost Consolidated Pty. Ltd. has been formed to market milk, ice cream, and milk products in Australia under the Foremost trade mark, in joint partnership with Consolidated Milk Industries Limited, Redfern, New South Wales.

Foremost manager in Australia will be CHARLES VAN UMMERSEN. A. E. MCCARTNEY and E. G. MCCARTNEY are the Australian directors of Foremost Consolidated Pty. Ltd. A. E. MCCARTNEY is also chairman of Consolidated Milk Industries Limited.

Borden's to Exhibit

The Borden Company will have a 10,000-square foot exhibit at the 1964-65 New York World's Fair in the largest structure in the industrial area, the Better Living Building. This building will be built on a three and one-half-acre plot adjacent to the bus terminal entrance and on the main circulation horseshoe surrounding the Lagoon of Industry. The building will be elevated 12 feet above grade, allowing unrestricted passage under the building to gardens and a 26,000-square foot lake area (see photo of proposed structure).



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These are only a few of the features which make CP Continuous Freezers a profitable choice of quality-minded and cost conscious ice cream men.

Very truly yours,

H. L. Mitten

H. L. Mitten
Director of Technical Sales

HLM:jkw

J. L. Dutt Promoted

Appointment of J. L. DUTT, 428 Winnebago, Park Forest, Ill., as manager of the Himes Bros. Dairy Division of Beatrice Foods Co., at Dayton, Ohio, has been announced by J. F. HAZELTON, SR., executive vice-president of Beatrice Foods.

J. L. Dutt

Dutt, a veteran of 14 years with the company, will supervise all Himes Dairy operations in Dayton and branches in Middleton, Lebanon, and Springfield, Ohio, and Richmond and Connersville, Ind.

Bowman Dairy Company and Long Meadow Farms Win First Places

Bowman Dairy Company of Chicago and Long Meadow Farms of Durham, North Carolina, won first places for excellence of newspaper advertising in the 27th Annual Advertising Contest sponsored by the Milk Industry Foundation. Held in connection with the Foundation's annual convention, the Contest attracted 567 individual entries from 69 different companies.

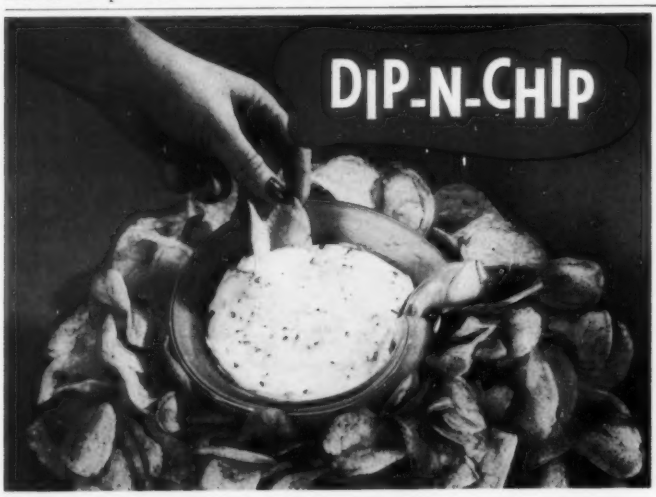
The Contest involved ten categories, ranging from newspaper advertising to billboards. Institutional ads, promotional material, recipe booklets, store cards, street car and bus cards, and dairy truck posters were other categories in the contest.

Frozen Semen Products Appoints R. W. Gabriel

R. W. GABRIEL has joined Frozen Semen Products as a technical representative in methods and procedures of freezing semen and the use of extremely low temperature equipment. Mr. Gabriel holds a Bachelor of Science degree from the University of Wisconsin and a Master of Science degree from the University of Minnesota. Both degrees were awarded in the field of Dairy Husbandry.

R. W. Gabriel

He also has had extensive experience in the commercial artificial breeding field, having been employed as a laboratory supervisor, service technician, field supervisor, and in the sales field. Invaluable experience in freezing



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and handling frozen semen has been acquired while working in the A.I. field.

U. S. Public Health Service Courses

The Public Health Service, through its Division of Environmental Engineering and Food Protection, will present two consecutive 1-wk. training courses, Laboratory Examination of Milk, February 12-16, 1962, and Laboratory Examination of Foods, February 19-23, 1962, in Cincinnati, Ohio.

The first course is designed for personnel in laboratories engaged in milk analyses and dairy-products examination. It presents the theory, laboratory techniques, and interpretation of tests used in evaluating the sanitary quality of milk and dairy products and includes laboratory practice in the special procedures employed in detection of bacterial groups or species of special significance in the control of milk quality.

The second course presents advance technical information for laboratory and supervisory personnel concerned with examination of foods for bacterial or chemical contaminants. It emphasizes methods, standards, and procedures applicable to a food-sanitation program, giving consideration to the relation of the laboratory to inspection, grading, and licensing of food establishments.

These courses follow Recent Developments in Water Bacteriology, February 5-9, 1962, for the convenience of those desiring to enroll in the three consecutive microbiological courses.

Instant Milk and Ice Cream Demonstrations in Germany

A return engagement for instant milk and a demonstration of the use of exportable dairy ingredients in ice cream making recently was scheduled for the Republic of Germany. These were at Fairs in Stuttgart and Hamburg, with Dairy Society International, American Dry Milk Institute, and American Dairy Association cooperating with the U. S. Foreign Agricultural Service. Dates were: Stuttgart, October 27-November 5; Hamburg, November 10-19.

MRS. MADGE LITTLE, nationally known home economist of American Dry Milk Institute and W. L. PHILLIPSEN, Assistant Administrator of Dairy Sociey International, will supervise the demonstration. FAS will be represented by W. L. SCHOLZ of the Dairy and Poultry Division.

Dairy Technology Societies

Central Illinois—The October meeting at the Jefferson Hotel, Peoria, featured Dr. F. A. Kummerow, professor of Food Chemistry, University of Illinois, with the subject: Questions and Answers on the Role of Fats in the Diet. Professor L. R. Fryman, extension

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specialist, Department of Dairy Science, University of Illinois, spoke at the November meeting on Milking Practices—Good and Bad—on Illinois Farms. The meeting was held at the Blue Mill Restaurant, Decatur. The December meeting will be in Champaign, and will honor the Illinois Dairy Products Judging Team.

Central Michigan—Carl Christianson, of the Cherry-Burrell Corporation, Chicago, was guest speaker at the October meeting, and brought members up to date on the vacuum treatment of fluid milk for the removal of off-flavors.

Detroit—The regular monthly meeting was held November 13 at Cregar's Pickwick House on Grand River. Speaker was Neil Angevine of the Meyer Blanke Company, St. Louis. His topic was cultured products and Cottage cheese.

Kansas—The September dinner meeting featured H. J. Breejen, Ex-Cell-O Corporation, Detroit, with the topic: What's New in Milk Packaging. Speaker for the November 13 meeting was Raymond Zeigler, Zeigler and Son, Topeka, speaking on The Manufacture of Cottage Cheese.

Kansas City Area—A social hour was held October 14 at Ward Parkway Country Club. The regular monthly meeting was on November 16, with D. R. Raney, V.C.M., speaking on Veterinary Medicine and You.

Metropolitan—The October 10 meeting welcomed new officers and had as guest speaker Robert W. Shafer, Eastern Manager, National Dairy Council, with the topic: Fats in the American Diet.

North Carolina—Dr. W. M. Roberts, head of the new Food Science and Processing Department at North Carolina State College, spoke at the October meeting on The Role of the Food Science and Processing Department. The November meeting had as speaker C. F. Rossignol, vice-president in charge of production and engineering, South Atlantic District, The Borden Company. His subject: Construction, Refrigeration, Loading and Unloading of Hardening Rooms.

Southern Illinois—At the September meeting Dr. Joseph Tobias, University of Illinois, spoke on the topic of New Trends in the Dairy Industry. Lyle Jackson, manager of Southern Illinois Breeders Association, spoke at the October meeting on Applying Cryogenics to the Field of Improving Dairy Cattle Through A.I.

Tri-State—Speaker at the October meeting was R. B. Beahm, Application Engineer, Taylor Instrument Company, Rochester, New York. Instrumentation in the Modern Dairy Plant was his subject. November meeting consisted of a panel discussion covering current items of interest on ice cream. Moderator was Dick Wagner, The Borden Company, Pittsburgh.

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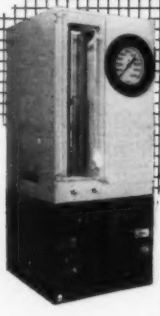

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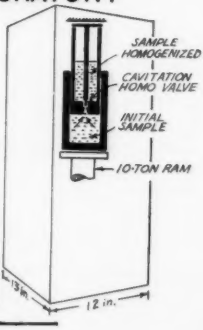

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Theses Completed

M.S. Degree:

MAURICE L. DAHNCKE—Milk composition and how it is affected by two types of rations, environmental temperature, and breed. Southern Illinois University.

GERALD R. KRAWCZYK—Effect of feeding stilbestrol and growth and breeding efficiency of dairy heifers. Southern Illinois University.

CHRISTIAN LEGAULT—The heritability of birth weight and its relationship with production in dairy cattle. University of Illinois.

WILLIAM RUFENER—Development of methods for determining the rumen microbial population in continuous culture. University of Illinois.

LARRY D. STEWART—High vs. limited level grain feeding for dairy cows. Southern Illinois University.

Ph.D. Degree:

FRANK N. DICKINSON—The additive dominance, and epistatic effects of six mutant genes on some quantitative characteristics of *Drosophila melanogaster*. University of Illinois.

ERIC W. HAHN—The effect of certain internal and external environments on reproduction and sex-ratio of Albino rats. University of Illinois.

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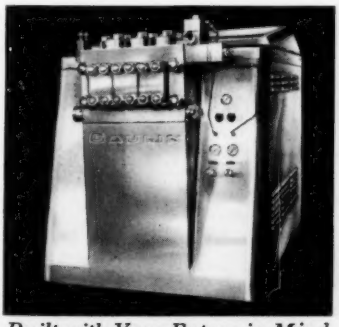

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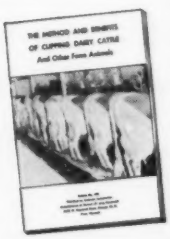

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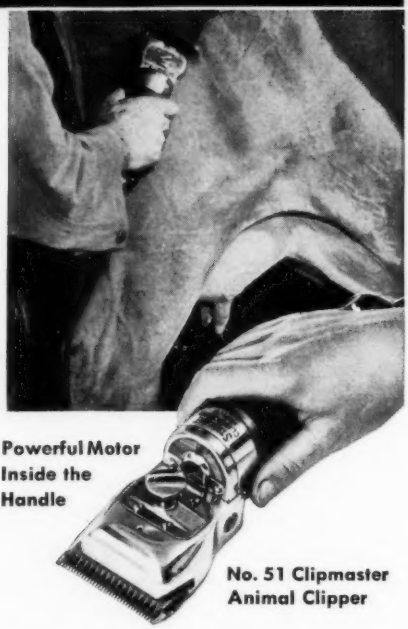

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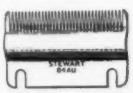

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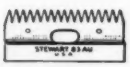

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DAIRY INDUSTRY

At the 56th Annual Meeting of the American Dairy Science Association at the University of Wisconsin in June, 1961, four competent authorities in education and research, government service, and in industry, presented papers about the opportunities for careers in Dairy Science and in the Dairy Industry.

These papers by Dr. Earl Weaver, Dr. H. F. Judkins, Dr. H. A. Herman, and Mr. D. L. Hume tell the story of opportunities to high school students and to college students who have not selected a major course of study. The material in these papers can be used as the basis of talks to dairy technology societies, and to state, federal, and commercial dairy organizations, chambers of commerce, and civic groups.

Every department charged with responsibility of training students in all land-grant colleges should have a supply of reprints of the four papers. They contain excellent material for recruitment purposes. Likewise, industry through its public relations activities may use these reprints for developing recruiting programs. Industry cannot afford not to encourage a flow of well-trained students into its various specialized branches.

The Garrard Press is holding the type for the four papers in order to give interested organizations an opportunity to order reprints. The four papers containing 11 pages will be charged at the 12-page rate. A reprint price schedule appears in all recent issues of the JOURNAL OF DAIRY SCIENCE.

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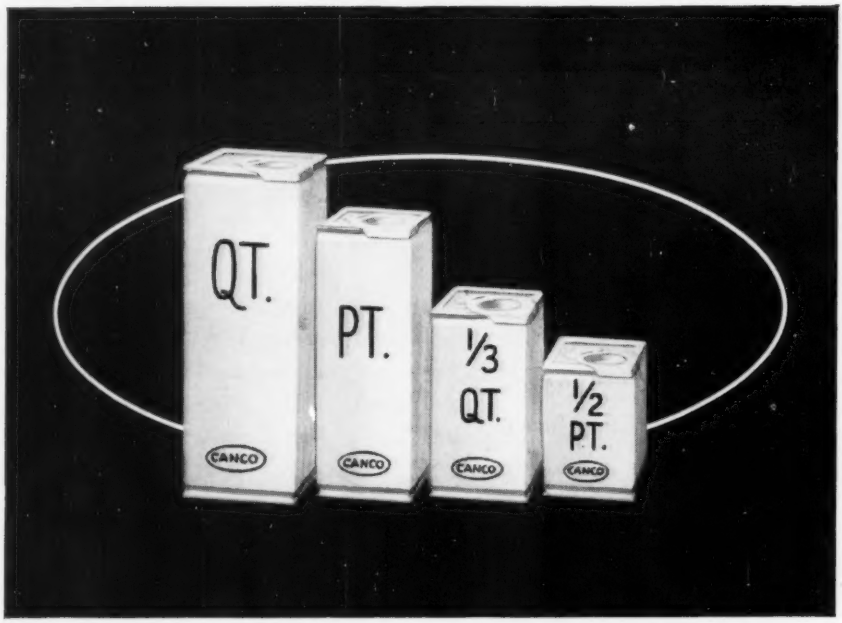

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RESEARCH PAPERS

EFFECT OF ISOLATION AND CRYSTALLIZATION ON THE ELECTROPHORETIC MOBILITY OF β-LACTOGLOBULIN AS DETERMINED BY PAPER ELECTROPHORESIS

M. J. PALLANSCH AND C. A. ANDERSON, JR.

Dairy Products Laboratory, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

SUMMARY

To study the electrophoretic properties of milk proteins under conditions as nearly similar as possible to those found in milk, a paper strip electrophoresis apparatus was designed which required small volumes of electrolyte and in which electro-osmosis could be substantially reduced. Using this apparatus, it was shown that β-lactoglobulin could be isolated from milk, crystallized, and returned to its native environment without grossly affecting its initial charge density.

Inherent in all protein isolation procedures is the supposition that the end product obtained has an exact counterpart in the native state of the system from which it was separated (14). With the increasing refinement of analytical techniques, many pure crystalline proteins have been shown to be heterogeneous in respect to either their chemical or their physical properties, or both (7, 10, 11, 13). One possible source of the observed micro-heterogeneity in crystalline protein preparations has been ascribed to the effects of the purification techniques employed in their isolation (4, 12). These facts tend to cast doubt upon the initial surmise that the crystalline proteins have exact counterparts in the system from which they were isolated. Comparison of the physical and chemical properties of proteins before and after isolation is difficult, or impossible, in most cases. However, some work has been done in this field.

Studies of the oxygen dissociation curves (2) and the spectral adsorption (1) of hemoglobin in the red blood cell and in systems containing isolated hemoglobin have indicated that this protein in its native environment has properties different from those observed after isolation has been effected.

In the course of a study of the protein interactions that occur in milk during the processing and storage of milk products, methods were developed for investigating the electrophoretic mobilities of the milk proteins in milk serum. A comparison of the electrophoretic mobilities of a sample of crystalline β-lactoglobulin and the native whey proteins of milk was made in protein-free whey. This paper reports results obtained during this study, which indicate that an isolation and crystallization technique now commonly employed to produce pure β-lactoglobulin does not affect the charge distribution on the surface of the molecules of this protein. Crystalline β-lactoglobulin is shown to have an electrophoretically similar counterpart in the native state of the system from which it was isolated.

Received for publication April 29, 1960.

Since the electrophoretic apparatus used in this study is somewhat different from types now in common use, its design and operation characteristics are described in some detail.

EQUIPMENT AND MATERIALS

A. Paper strip electrophoretic apparatus. The apparatus used in this investigation is a variation of the simple design of Kunkel and Tiselius (9), in which an electrical potential is applied through large open electrode vessels to the ends of a buffer-dampened paper strip sandwiched between two pieces of plate glass. This design was modified to eliminate the bulky electrode compartments and minimize the amount of electrolyte used for an analysis. The electrical potential was applied to the paper strip through reversible silver–silver chloride electrodes embedded in blocks of agar containing KCl.

The apparatus was constructed from two blocks of Lucite,[1] each 24 by 4 in. One block was ¾-in. thick, the other ½-in. thick. Two rectangular electrode compartments 4⅛ by 2⅛ in. were milled out of the ends of the thickest block. The distance between the inside edges of the compartments was 17⅜ in. Electrodes consisting of four ⅜-in. o.d. coils of $\frac{1}{16}$-in. silver wire were placed in each compartment and connected through a $\frac{1}{16}$-in. hole drilled through the side of each well to terminals mounted on the side of the block. After the electrodes were electrolytically plated with silver chloride, the wells were filled to the top with a hot 2% agar solution containing 1% KCl. On cooling, the agar solidified around the silver–silver chloride coils to form a stable reversible electrode system.

To maintain the apparatus at constant temperature during operation, a water jacket 23 by 5 by ¼-in. was cemented onto the outside face of each block.

The apparatus was held together by six 2-in. C clamps.

Direct current was obtained from a Spinco Duostat [1] power pack.

Figure 1 is a photograph of the apparatus as used in the described experiments.

B. Protein-free whey. A solution having very nearly the some composition and ionic activity as the phase in which the milk proteins are found in their native state, was prepared by dialyzing 200 ml. of distilled water against 40 liters of skimmilk. The dialysis was carried on for five days with intermittent agitation at 5°C. Ten milliliters of toluene were added to the milk before dialysis, to prevent bacterial growth.

C. Native whey protein concentrate. Skimmilk was centrifuged in the A Rotor of a Model E Spinco [1] Ultracentrifuge for 60 min. at 57,000 × G and 23°C., to remove colloidal caseinates. The supernatant solution was further centrifuged in the same rotor for an additional 6 hr. at 150,000 × G and 23°C., to concentrate the whey proteins. After centrifugation, all material except the pellet and bottom 1 ml. of solution was removed from the tubes. The pellets were broken up into the residual solution in the tubes and allowed to stand at room temperature for approximately ½ hr. The material was collected and centrifuged in a clinical centrifuge for 15 min. The clear supernatant solution

[1] The mention of trade names in this paper is for identification and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

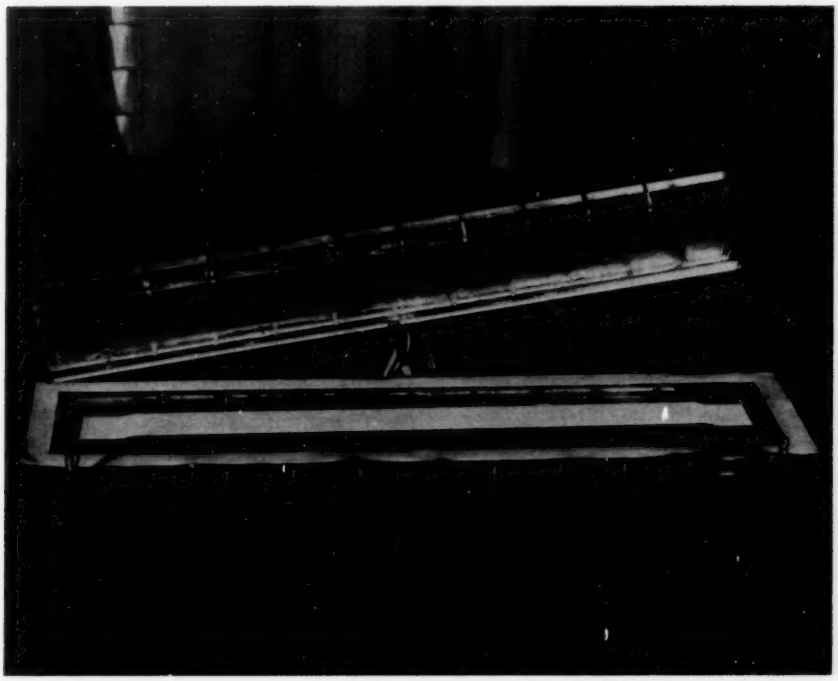

FIG. 1. Photograph of paper electrophoresis apparatus equipped with reversible silver–silver chloride electrodes imbedded in agar gel.

representing concentrated whey proteins in unmodified whey was held under refrigeration in an atmosphere of toluene for electrophoretic analysis. These solutions were found to be relatively unstable, and were not stored for periods in excess of three days.

D. Purified β-lactoglobulin. Crystalline β-lactoglobulin was prepared according to the method of Gordon *et al.* (6). After triple recrystallization the crystals were collected by centrifugation and lyophilized. The dried material was stored under refrigeration. Subsequent analysis showed this preparation to be electrophoretically homogeneous in phosphate buffer at pH 7.5.

EXPERIMENTAL METHODS

The electrophoretic characteristics of the proteins used in this study were determined on strips of Whatman No. 1 filter paper 22 in. long and ranging from ½ to 3 in. in width. The type of strip selected was wet with the solution in which the analysis was to be carried out. Approximately 2.5 ml. of solution were required for each inch of strip width. Excess fluid in the strip was removed by blotting between sheets of filter paper. The damp strip was then placed on the surface of the electrode-containing plastic block and the ends of the strip

were brought into contact with the agar surfaces of the electrodes. Electrical contact between the agar and the paper strip was established by adding an additional drop of electrolyte to each end of the strip. A sample of the protein solution to be analyzed was placed on the paper near the center of the strip in the form of a spot or a line, using either a micropipette or a Spinco [1] applicator. Sample size used ranged from one lambda to ten lambda, depending on the protein concentration in the sample analyzed.

Evaporation of water from the strip during electrophoresis was controlled by placing a Teflon [1] gasket ¼-in. wide and approximately 0.01 mm. thick around the outer edge of the block. The strip was then covered with the second Lucite [1] block and two blocks were clamped together, using six 2-in. C clamps. Uniform pressure on the strip is essential for proper operation.

The apparatus was equilibrated by allowing water of the desired temperature to circulate through the jackets for a 15-min. period before starting the analysis. Water temperatures ranging from 10 to 23°C. were used during the study. Direct current was then passed through the strip long enough to achieve sufficient protein migration on the strip, the intervals ranging from 1 to 6 hr. On completion of the runs the strips were removed from the apparatus, dried in a circulating air oven for 20 min. at 130°C., and stained with bromphenol blue (9).

The electrodes were reversed in polarity after each run, to maintain their silver chloride coating. If the buffers used in the analysis did not contain chloride, the electrodes were regenerated electrolytically after each week of operation.

The operational efficiency of the apparatus was determined by comparing strips obtained by its use with those obtained using a commercial model of the Durrum type apparatus (5). The fact that the new apparatus was water-cooled enabled much higher potentials—up to 720 volts—to be placed across the strip. Therefore, equivalent migration and resolution could be obtained in much shorter time than required for the Durrum type apparatus.

Restriction of electro-osmotic flow of water in the strip was studied by operation of the apparatus in positions other than horizontal.

The mobility of the crystalline β-lactoglobulin dissolved back into whey was compared with the mobility of the whey proteins not removed from their native environment. Whatman No. 1 paper strips were wet with protein-free whey, and samples of crystalline β-lactoglobulin dissolved in whey and native whey protein concentrates were allowed to migrate through the strips under the influence of identical electrical potentials. The rate of migration of the two materials in the native environment of the milk proteins was compared.

To determine the effect of the presence of colloidal caseinates on β-lactoglobulin in its native environment, small amounts of the caseinates initially centrifuged out of the milk were added back to the native whey-protein concentrate before electrophoretic analysis in protein-free whey.

RESULTS

During preliminary studies it was found that whey protein mixtures could be resolved and the electrophoretic mobilities of proteins validly compared by

use of paper electrophoresis apparatus equipped with reversible electrodes imbedded in a gelled electrolyte.

With an apparatus of this type, the electro-osmotic flow of water in the paper strips (3, 8) could be greatly reduced and, in most cases, completely eliminated. This was accomplished by operating the apparatus in a vertical position, allowing the force of gravity to act against the electro-osmotic forces operating in the paper strip. Using lactose as a neutral marker compound, the extent of electro-osmotic flow in the strip was determined. If the strips were wet with veronal buffer pH 8.6, ionic strength 0.10, the water moved 0.6 cm. per hour towards the anode, with a potential of 700 volts impressed across the strip oriented in a horizontal plane. Under the same conditions, with the strip oriented in a vertical position with the cathode up, the rate of marker migration was 1.3 cm. per hour. With the strip in a vertical position, with the cathode at the lower end of the strip, no electro-osmotic effects were observed.

The low electrolyte requirement of the apparatus also made possible the determination of the mobility of some of the whey proteins in protein-free whey. Crystalline β-lactoglobulin dissolved in protein-free whey was found to migrate through whey exactly as fast as the fastest component in the native whey protein concentrate, when both were subjected to fields of equal electrical potential, as illustrated in Figure 2.

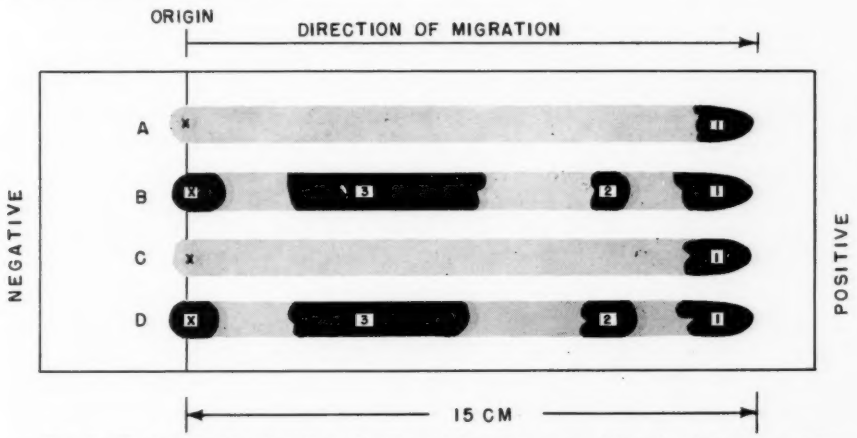

FIG. 2. Electrophoretic migration of milk proteins on a paper strip saturated with protein-free whey. 5λ of 4% solution of crystallized β-lactoglobulin in protein-free whey were spotted at x on A and C and 5λ of a native whey protein concentrate of approximately 8% protein were spotted at x on B and D. Diagram indicates results after 6 hr. of electrophoresis at 20°C., with a potential of 700 volts across ends of strips. Spots numbered one are β-lactoglobulin. Spots 2 and 3 are unidentified whey proteins. Shaded areas represent trails of proteins adsorbed on strip.

Adding the caseinate complex back to the native whey protein concentrate caused a slight shift in the distribution of the fastest component towards the point of origin, as can be seen in Figure 3. The leading edge of the zone con-

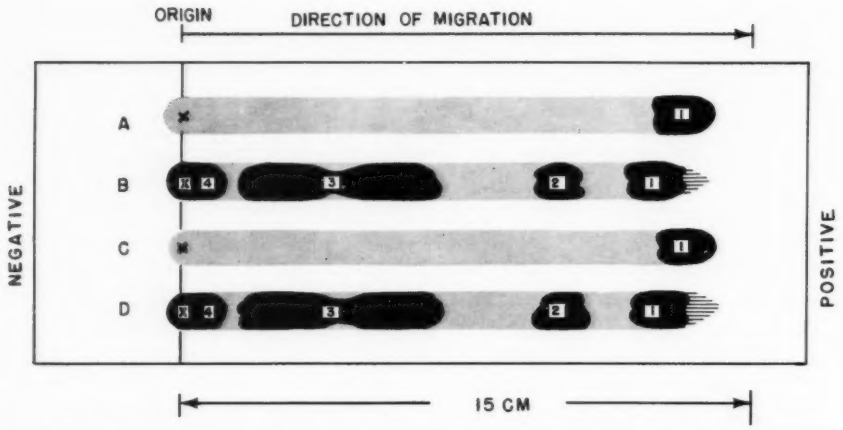

FIG. 3. Electrophoretic migration of milk proteins on a paper strip saturated with protein-free whey. 5λ of a 4% solution of crystallized β-lactoglobulin in protein-free whey were spotted at x on A and C and 5λ of a native whey protein concentrate of approximately 8% protein plus 5λ of a 4% solution of casein in whey were spotted at x on B and D. Diagram indicates results after 6 hr. of electrophoresis at 20°C., with a potential of 700 volts across ends of strips. Spots numbered one are β-lactoglobulin. Spots 2 and 3 are unidentified whey proteins. Spots 4 are unidentified whey proteins plus casein. Shaded areas represent trails of proteins adsorbed on strip. Parallel lines indicate zones of low protein concentration.

taining the fastest component maintained the rate of migration noted before the addition of the caseinate. The amount of fast-moving protein shifted toward the origin depended on the diameter of the caseinate spot which remained at the point of application during the analysis. This possibly indicates that the β-lactoglobulin might have been retarded by a reversible protein-protein interaction with the caseinate as it migrated across the spot of application at the start of analysis.

DISCUSSION

Observations made with the equipment described in this report lend some further support to the supposition that β-lactoglobulin isolated from milk in a crystalline condition has undergone no radical change in physical properties. Although electrophoretic mobilities offer only one criterion for comparison, it is noteworthy that the electric charge density of the isolated protein is unchanged after return to its native environment.

Evidence of interaction between β-lactoglobulin and the casein micelles observed on the whey-saturated strips does not mean this situation exists in milk. The caseins are bound to the paper strip with forces sufficiently high to possibly change their physical structure to the point where interaction with β-lactoglobulin will occur.

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COMPARATIVE HYDROLYSIS OF PRIMARY AND SECONDARY ESTER POSITIONS OF MODEL COMPOUNDS OF GLYCEROL BY MILK LIPASES [1]

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SUMMARY

Both positional isomers of dichloropropanol, diethoxy propanol and propanol, were esterified with butyric and palmitic acid and the resulting esters subjected to milk lipase action. Since insignificant hydrolysis occurred when the fatty acid was esterified in the 2 position, milk lipase probably is specific for the primary ester position.

We have noted in previous studies that although milk lipases preferentially hydrolyzed the primary ester positions of milk and synthetic triglycerides, some apparent hydrolysis of the secondary ester position also occurred (1, 3, 4). Under the conditions of the previous work, we were unable to determine whether the apparent hydrolysis of the secondary position was due to actual hydrolysis or to acyl migration of the secondary ester to the primary position and subsequent hydrolysis. Furthermore, it is not convenient to demonstrate primary ester position specificity when the 2-position is esterified with some short-chain acids, as would be the case in milk fat, because of the expected higher acyl migration rate of these short-chain acids.

Since acyl group migration accompanying lipolysis prevents demonstration of absolute specificity for both long- and short-chain fatty acids esterified in the primary position, model compounds were synthesized in which acyl group migration could not occur because two of the positions were blocked by hydrogen, ethoxy (ether), or chloro groups. These model compounds were then subjected to milk lipase action, to determine whether this enzyme system shows absolute specificity for the primary ester positions.

EXPERIMENTAL PROCEDURE

Synthesis of compounds. (a) 1,3- and 1,2-Diethoxy propyl butyrate and palmitate. 1,3-Diethoxy glycerol was purchased and carefully fractionated with a spinning-band distillation column, yielding both 1,3- and 1,2-diethoxy glycerol. These compounds were acylated with butyryl or palmitoyl chloride in the presence of pyridine and chloroform, then distilled. Purification of the fractions was followed by gas-liquid chromatography on Apiezon and diethylene glycol succinate columns.

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(b) 1,3- and 1,2-Dichloro propyl butyrate and palmitate. These compounds were prepared as described in (a), except that the starting materials were 1,3- and 1,2-glycerol dichlorohydrin.

(c) Propyl and isopropyl butyrate and palmitate.

These compounds were made as described in (a), except that propyl and isopropyl alcohols were the initial materials. All of the compounds synthesized, with the exception of the 1,2-diethoxy and 1,2-dichloro compounds, were obtained in very high purity and yielded a single peak when chromatographed. The 1,2 isomers could not be purified by distillation because they boiled at a temperature only slightly higher than that of the 1,3 isomer. All of the compounds synthesized were liquids at the incubation temperature (37° C.).

Digestion conditions. One gram of substrate was emulsified into water, with a Waring Blendor: 3 g. of lyophilized skimmilk was added, and the mixture was made up to 20 ml. The solutions were then adjusted to pH 7.0 in all cases with ammonium hydroxide solution. These reaction mixtures were incubated at 37° C., with shaking for from 30 to 90 min. Controls were prepared by momentarily heating the raw skimmilk solution to boiling before addition of substrate. Incubation of the raw skimmilk did not give any increase in acid and was, therefore, run only occasionally. Three digestions were carried out on each substrate.

Analysis of hydrolytic products. After incubation, the free fatty acids were determined as described by Harper *et al.* (2), and the rate of hydrolysis was expressed as millimoles of acid produced per hour.

RESULTS AND DISCUSSION

The relative rate of hydrolysis of each substrate is given in Table 1. In each instance when the fatty acid was esterified in the 2 position, insignificant hydrolysis occurred. However, when the fatty acid was esterified in the 1 position, hydrolysis occurred in all but one case. 1,2-Diethoxy-3-propyl butyrate and the corresponding palmitate derivative were hydrolyzed rather slowly. Since these compounds were contaminated with the 1,3 isomer, as stated previously, the slow rate may have been due to low substrate concentration or inhibition by the 1,3 compound. n-Propyl palmitate was not hydrolyzed, although monoolein

TABLE 1

Rate of hydrolysis of model compounds by milk lipase[a]

Propyl butyrate		Rate[b]	Propyl palmitate		Rate[b]
Diethoxy	1,3	0	Diethoxy	1,3	0.04
	1,2	0.70		1,2	0.42
Dichloro	1,3	0.05	Dichloro	1,3	−0.05
	1,2	3.30		1,2	.....
Iso		−0.07	Iso		0.04
Normal		2.24	Normal		0
1-Monoolein		3.60			

[a] Reaction conditions: 12 g/100 ml. of raw skimmilk and 4 g/100 ml. of each substrate were incubated at pH 7.0 and 37° C.

[b] Millimoles of acid liberated per hour.

was readily digested, which may be explained by the observation of Schönheyder and Volquartz (5), that hydroxyl groups adjacent to primary ester positions enhanced activity of pancreatic lipase. Milk lipase seems to hydrolyze n-propyl butyrate readily, although this may be indicative of esterase activity.

Isopropyl butyrate was not digested, while n-propyl butyrate was attacked. This is probably the best evidence for absolute specificity of milk lipase for the primary position, since primary butyrate esters are readily attacked. While unequivocal proof of the absence of activity for secondary ester positions is not obtainable, it seems reasonable to conclude that milk lipase, which may include simple esterases, shows little specificity for secondary ester positions.

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FATTY ACID COMPOSITION OF THE DIGLYCERIDES FROM LIPOLYZED MILK FAT[1]

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SUMMARY

The diglycerides in eight samples of lipolyzed milk were isolated by silicic acid column and thin-layer chromatography and identified by infrared spectrophotometry. The average recovery of diglycerides was 93.3%. Controls contained negligible quantities. Fatty acids, identified by gas-liquid chromatography, were: palmitate, 29.5%; oleate, 24.4%; myristate, 13.2%; stearate, 8.4%; and at least 16 others commonly found in milk fat. The fatty acid composition of the diglycerides was different in some respects from that in the intact control milk fat. About 70% of the monoglycerides was recovered and similar data are reported for these compounds.

Milk lipases digest synthetic triglycerides in a step-wise sequence similar to that of pancreatic lipase, producing free fatty acids and mono- and diglycerides (4). Monoglycerides and free fatty acids are also produced by the action of milk lipases on milk fat. If the action of milk lipase on milk fat and on synthetic triglycerides is similar, then diglycerides should likewise be present in lipolyzed milk fat. However, evidence for the presence of diglycerides has been circumstantial. For example, the quantity of free fatty acids found in lipolyzed milk fat is usually much larger than can be accounted for by assigning two m*M* of free fatty acid to each m*M* of monoglyceride present (7, 9). If it is assumed that milk lipases produce very little glycerol from monoglycerides in a manner similar to pancreatic lipase (14), then the free fatty acids not accounted for by the monoglycerides must have been released from triglycerides to form diglycerides.

The recovery and analysis of diglycerides from lipolyzed milk fat have been hampered by the lack of an analytical method for determining small quantities of diglyceride in the presence of other glycerides. The complexity of milk fat has made it difficult to obtain diglycerides from this source by silica gel column chromatography. However, further fractionation of the material obtained from the silica gel column by thin-layer chromatography (13, 17) has enabled us to isolate the diglycerides from lipolyzed milk. In this paper we describe the isolation of diglycerides from lipolyzed milk fat by silica gel column and thin-layer chromatography, analyses of the glycerides by infrared spectrophotometry, and identification of the component fatty acids by gas-liquid chromatography (GLC).

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EXPERIMENTAL METHODS

Sample preparation. Employing eight samples, lipolyzed milk was prepared by mixing raw and homogenized milks in equal parts followed by storage at 4° C. for 48 hr., and control milk was similarly prepared, except that the raw milk was first either boiled or treated with acid to inactivate lipase. Acid degree values (5) and monoglyceride contents (9) were determined on the fat extracted by the silica gel method (5) from 50-ml. portions of each lipolyzed and control milk. In addition, the fat from another 50-ml. portion was used as a source of di- and monoglycerides. Free fatty acids were removed from this portion by passage through an ion exchange resin (2), the solvent removed from the lipids with a flash evaporator, and the lipids weighed. The lipids were then applied to a silica gel column (6), to concentrate the diglycerides. The first fraction from the column, eluted with 6% ethyl ether–94% Skellysolve B, contained most of the triglycerides. The second fraction from the column, eluted with ethyl ether, contained some triglyceride, cholesterol, diglyceride, and monoglyceride. Approximately 30% of the monoglycerides remained on the silica gel column.

Determination of glycerides. To determine the glyceride composition of the two fractions from the silica gel column, thin-layer chromatography (TLC), with the aid of reference compounds (13, 17), was employed. Since, in preliminary experiments, cholesterol interfered with subsequent separation of diglycerides by preparative TLC, it was removed with either tomatine (10) or digitonin. The second silica gel column fractions, dissolved in a minimal amount of chloroform, were streaked on thin-layer silicic acid plates for preparative TLC. A solvent system of petroleum ether (30-60°), ethyl alcohol, and acetic acid (90:10:1 by volume) was used. The resulting four or five glyceride bands on the TLC plates were made visible either by ultraviolet light or by spraying one edge of the plate (13), outlined with a pointed spatula, scraped off the glass plate, extracted with chloroform-methanol (90:10), and filtered into tared evaporating flasks. Difficulty was experienced at this point with the passage of some silicic acid through the filter paper. An effort was made to obtain precise weights of the fractions from four of the eight samples by carefully rinsing the lipids from the particles with chloroform. This was not done with the first four samples and these data were rejected. After removing the solvents with a rotary evaporator and weighing the residue, infrared spectra were obtained on the material extracted from each band on the TLC plate. Monoglyceride concentrations were also estimated colorimetrically (9). Comparison of the infrared spectra with published results (15), and with those obtained from reference compounds purified by TLC, indicated that the first band up from the line of application on the TLC plate was monoglyceride; the second, diglyceride; the third, a mixture of di- and triglycerides; and the fourth, which ran with the solvent front, triglyceride. The third band was rechromatographed by TLC as above and the diglycerides obtained were combined with those from the second band. The combined diglycerides were identified as such by infrared spectro-

photometry, using absorption at 9.6 μ as one of the criteria (15). Recoveries of the total lipids applied to the plates averaged 92.2%.

Gas liquid chromatography. The control milk fat and the di- and monoglycerides obtained from the bands on the TLC plates were each converted to methyl esters by refluxing with dry methanol and to butyl esters by refluxing with butanol, employing sulfuric acid as a catalyst. These esters were separated by gas-liquid chromatography, using an Aerograph (A-90-C) instrument. A diethylene glycol succinate column on Gas-Chrom P[3] (15%) was employed for the methyl esters and an Apiezon L column on glass beads (0.25%) coupled with temperature programming was used for the butyl esters. Identifications were made by comparisons with authentic reference compounds.[3] Minor peaks were tentatively identified by plots of retention time versus chain length and in some cases it was not possible to differentiate between branched-chain and unsaturated fatty acids. Quantities of fatty acid esters were determined by measuring areas on the charts with an integrator built into the recorder. The butyl esters were calculated as methyl esters and are reported as such. Butyl esters were prepared so that some of the difficulties experienced in recovering methyl butyrate and caproate from the interesterification mixture would be avoided.[4]

RESULTS AND DISCUSSION

The quantities of the mono-, di-, triglycerides, and fatty acids from four of the samples analyzed in this study are presented in Table 1. Diglycerides were

TABLE 1

Acid degree values and partial glyceride contents of control and lipolyzed milk fats

	Milk sample (No.)			
	1	2	3	4
	(mM per 100 g. fat)			
Control FFA	2.42	1.61	1.70	1.71
Control monoglyceride	0.21	0.22	0.28	0.28
Control diglyceride	0.87	0.47	0.90	0.58
Lipolyzed FFA	21.24	11.66	11.69	12.00
Lipolyzed monoglyceride	4.73	3.06	3.03	3.04
Lipolyzed diglyceride				
Molecular weight [a]	564	558	550	558
Theoretical content [b]	11.78	5.54	5.63	5.92
Recovered	9.62	5.34	5.20	6.06
Per cent recovery	81.7	96.4	92.4	102.4
FFA resulting from mono- and diglyceride formation	19.08	11.46	11.26	12.14

[a] Calculated from fatty acid content.

[b] Theoretical diglyceride content = mM of FFA per 100 g. fat − 2(mM of monoglyceride per 100 g. fat).

a product of lipolysis by milk lipase, since the diglyceride contents of the lipolyzed samples were much greater than the content of the control samples. The theoretical quantities of diglyceride were calculated by assuming that all

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[4] Details of the butyl ester procedure are to be published in the near future.

of the free fatty acids not accounted for by the monoglyceride content must have been derived from triglycerides to form diglycerides. Therefore, since each millimole of monoglyceride accounts for 2 m*M* of fatty acid, the theoretical diglyceride content equals millimoles of FFA per 100 g. fat minus twice the millimole of monoglyceride per 100 g. fat. The molecular weights of diglycerides were calculated from the fatty acid compositions. The millimoles of diglycerides recovered were calculated from the molecular weight and the amount recovered. In making these calculations we assumed that no monoglyceride was lipolyzed to form glycerol. In subsequent experiments, we discovered that milk lipases will hydrolyze 1-monoglycerides. However, our assumption is still valid because milk lipases attack the 2-position of milk triglycerides sparingly, if at all (4, 7), and the monoglycerides produced by the action of milk lipases are the 2-isomers, which change slowly to the 1-isomer by acyl migration (7, 9). About 75% of the monoglycerides isolated from the thin-layer plates was the 2-isomer. The results in Table 1 support this assumption, since the mono- and diglycerides account for most of the FFA when the FFA calculated as having been derived to form mono- and diglycerides are compared to the total lipolyzed FFA. The calculations do not apply to the control samples, there being more diglyceride present than can be accounted for in this way. This discrepancy may be due to diglycerides left over from fat synthesis.

With TLC, it is possible to detect 0.1% of a single component in a mixture of mono-, di-, and triglycerides (17). The ability of TLC to separate lipid classes, coupled with identification of the diglycerides by infrared spectrophotometry, assured us that the diglycerides were relatively pure.

The monoglycerides in the second fraction eluted from the silica gel column, when further purified by TLC and estimated colorimetrically (9), contained about 320 m*M* of monoglyceride per 100 g. fat, which approached 100% purity when the molecular weights obtained from the fatty acid composition data were used in the calculations. This remarkable purity illustrates the value of thin-layer chromatography.

The fatty acid composition as methyl esters of the original triglycerides, and the di- and monoglycerides resulting from milk lipase action, are presented in Table 2. The fatty acid indentification is given by the number of carbons, followed by the number of double bonds, if any (3). The trend was for 10:0, 12:0, 14:0, 16:0, and 16:1 to exist in higher concentrations in both the di- and monoglycerides. In contrast, 18:0 and 18:1 were found in lower concentrations in the partial glycerides, except for 18:1 in the diglycerides. Results in Table 2 agree well with those of McCarthy *et al.* (12), Patton *et al.* (16) and, in some respects, with the data of Ast and Vander Wal (1). All of these studies employed pancreatic lipase to digest milk fat. The information on the composition of milk monoglycerides we obtained using the allyl ester procedure (8) also agrees fairly well with similar data in Table 2.

The apparent specificity of milk lipase for several of the fatty acids (Table 2) may be due to positioning of the acid and preferential hydrolysis of primary ester linkages (4). For example, the apparent specificity for butyric acid can

be explained by the almost exclusive positioning of this acid on the 1,3 positions of triglycerides (11). Therefore, fatty acid specificity probably can not be detected from Table 2 or similar data, unless the relative amounts of the fatty acid on the primary and secondary positions are known.

TABLE 2

Fatty acid compositions of milk triglycerides and the di- and monoglycerides resulting from lipolytic action [a]

Acid [b]	Triglyceride	Diglyceride	Monoglyceride [c]
	(% methyl esters by weight)		
4:0	3.3	1.3	0.5
6:0	2.3	2.1	1.4
8:0	1.7	1.5	1.5
10:0	3.1	3.5	4.8
11:0 [d]	0.4	0.4	0.1
12:0	3.3	4.0	5.2
13:0	0.2	0.3	0.1
14:Br [d]	0.2	0.3	0.1
14:0	11.3	13.2	17.5
14:1 [d]	1.8	1.9	0.7
15:0	1.3	1.9	1.0
15:1 [d]	0.4	0.9	1.3
16:0	29.1	29.5	31.2
16:1	2.4	2.9	8.1
17:0	0.8	0.4	0.5
18:Br [d]	0.4	0.7	1.2
18:0	11.5	8.4	7.5
18:1	23.8	24.4	21.6
18:2	2.0	1.7	0.4
Unknown	0.7	0.7	0.3

[a] Average of eight samples.

[b] The first figure designates the number of carbons and the second the number of double bonds.

[c] Not recovered completely.

[d] Tentative identification by plots of retention times versus chain length.

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RESISTANCE OF CERTAIN BACTERIA TO COTTAGE CHEESE COOKING PROCEDURES

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SUMMARY

Heat resistance in Cottage cheese whey at pH 4.6 was determined for selected strains of *Pseudomonas fragi, Escherichia coli, Streptococcus cremoris,* and *Streptococcus lactis.* Strains were selected for study on the basis of high heat resistance. Survivor curves in whey at various temperatures and thermal resistance curves were calculated. The time-temperature treatments used for cooking Cottage cheese curd in seven representative California plants and one hypothetical procedure were evaluated as to lethality for each of the selected cultures. Cooking procedures that did not have a maximum temperature of at least 130° F., and a holding time of at least 18 min. at 130° F., proved unsatisfactory.

Three of the most important defects of Cottage cheese are spoilage from psychrophilic bacteria, coliform bacteria, and sourness (6-8). Numerous reports deal with preventing contamination with psychrophiles and coliforms, and preventing microorganisms from producing defects. The development of sourness is related to lactic streptococci used in manufacturing the curd. Sourness may follow storage of cheese at 50° F. and above, inadequate cooking, or inadequate washing of the curd.

Cottage cheese is cooked to expedite expulsion of whey from the cubes of curd. Heating also improves keeping quality. The various cooking procedures used, developed from practical experience over many years, differ considerably in total heat. Data on the resistance of certain important bacteria to the heat applied are inadequate for use as a guide in developing proper procedures. Adequate data are particularly needed now, in view of current interest in mechanization of curd production, recent attention to decreasing the time required in making curd by adding starter stimulants that expedite the expulsion of whey (11), and the constant demand for Cottage cheese with better keeping quality.

An investigation was conducted to determine the heat resistance of selected psychrophiles, coliforms, and lactic streptococci, and to use the data in evaluating representative cooking procedures in present use.

METHODS

Culture selection. Cultures were selected on the basis of heat-resistance determinations in pasteurized whey at pH 4.6. The heat-treatment procedure used is described below. Coliforms and lactic streptococci were tested at 125° F.; psychrophiles were tested at 115° F. The selection tests involved 21 single-strain cultures of *Streptococcus cremoris,* three of *Streptococcus lactis,* six of *Pseudomonas fragi,* two of *Pseudomonas viscosa,* three of *Escherichia coli,* three of

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Aerobacter aerogenes, and seven coliform cultures recently isolated and not completely identified. From each group of bacteria were chosen one or two of the most heat-resistant strains.

Lactic streptococci were routinely propagated in litmus milk at 72° F., coliforms on plate-count agar medium at 95° F., and psychrophiles on plate-count agar medium at 77° F. Immediately before being tested, lactic streptococci were grown in litmus milk 16 hr. at 90° F.; coliforms were grown 40 hr. in litmus milk at 95° F.; and psychrophiles were grown 64 hr. in litmus milk at 77° F. These cultures were removed from the incubators and refrigerated 5 hr. before being used in securing data for survivor curves.

Heat treatment. Strains of bacteria were heat-treated in a constant-temperature water bath. Whey at pH 4.6 was obtained from a vat of Cottage cheese, dispensed in 6-oz. bottles, pasteurized 30 min. at 145° F., and stored frozen until used. One milliliter of litmus milk culture was added to 25 ml. of whey. The mixture was agitated, dispensed into three-dram screw-cap vials (2 ml. per vial), and placed in an ice-water bath. At the desired time, six to eight of the vials, in a mechanical agitating device, were submerged in the water bath. One vial was removed to the ice-water bath after the predetermined come-up time had elapsed; the others were removed at prearranged time intervals.

An average come-up time, to within ½° F. of each temperature used, was determined with a Leeds and Northrup potentiometer for 2-ml. samples of whey in three-dram vials. Come-up times varied from 2.5 min. at 113° F. to 3.5 min. at 137° F. To prevent a drop in bath temperature, a booster heater was turned on immediately before the samples of culture-whey mixture were placed in the water bath.

Numbers of surviving streptococci were determined by plating on modified trypticase soy agar (9). Plates were incubated 72 hr. at 90° F. Coliforms and psychrophiles were plated on plate-count agar medium. Coliform plates were incubated 48 hr. at 95° F.; psychrophile plates were incubated 72 hr. at 77° F. Duplicate plates were poured for each dilution; the resulting counts were averaged.

RESULTS

Heat resistance in skimmilk, whey at pH 4.6, and whey at pH 6.6. Heat resistance was compared in skimmilk, whey at pH 4.6, and whey adjusted to pH 6.6, using *P. fragi* (D) and *P. viscosa* (A). The results are in Table 1. Both cultures were considerably more resistant in skimmilk and whey at pH 6.6 than in whey at pH 4.6. Neither was more resistant in skimmilk than in whey at pH 6.6.

Selection of cultures. Experiments revealed that all *S. lactis* strains tested were more heat-resistant than the *S. cremoris* cultures. Selected for subsequent experiments were Strain T of *S. lactis* and Strain C of *S. cremoris*.

Initially, *E. coli*, Strain E, and *A. aerogenes*, Strain C, were chosen as the most heat-resistant strains of these species. Study with *A. aerogenes* was discontinued after the average D value for Strain C at 125° F. was found to be only 1.89 min., compared to 11.66 min. for *E. coli* (E).

TABLE 1

D values (in minutes) calculated from numbers of bacteria that survived laboratory heat treatments at 120° F. in skimmilk, Cottage cheese whey at pH 4.6, and whey adjusted to pH 6.6

Culture	Trial	Skimmilk	Whey at pH 6.6	Whey at pH 4.6
Pseudomonas fragi (D)	1	20.53	30.12	3.95
	2	28.09	30.21	4.73
	3	23.36	31.06	3.97
	4	15.70	34.72	3.65
	5			4.20
	6			4.44
	Average	21.92	31.53	4.16
Pseudomonas viscosa (A)	1	2.99	3.41	0.45
	2	5.55	2.60	0.36
	3	3.37	6.36	0.78
	4	2.76	3.42	
	Average	3.67	3.95	0.53

TABLE 2

D values (in minutes) calculated from numbers of bacteria that survived laboratory heat treatments in Cottage cheese whey at pH 4.6

		Temperature (° F.)				
Culture	Trial	113	119	125	131	
Pseudomonas fragi (B)	1	15.84	4.85	3.72	1.27	
	2	13.53	5.65	4.46	1.49	
	3	13.16	5.85	5.99	1.79	
	4	17.79	5.65	3.97	1.23	
	5	13.70			1.67	
	6	12.05			1.60	
	Average	14.35	5.50	4.53	1.48	
		113	116	119	122	125
Streptococcus cremoris (C)	1	18.66	8.74	4.18	3.47	1.56
	2	20.28	9.27	4.44	3.45	1.69
	3	14.16	8.80	4.33	3.76	1.68
	4	18.59	8.04	4.85	3.73	1.58
	5	15.75	8.34	4.83	2.60	
	6		7.82		2.66	
	Average	17.49	8.50	4.53	3.28	1.63
		122	125	128	131	
Streptococcus lactis (T)	1	22.73	9.62	7.49	3.88	
	2	21.69	12.56	7.85	3.30	
	3	11.67	9.90	7.65	3.07	
	4	21.23	9.80	6.48	4.58	
	5	12.74		5.74		
	6	20.24		6.54		
	Average	18.38	10.47	6.96	3.71	
		125	128	131	134	137
Escherichia coli (E)	1	9.31	6.49	5.00	1.88	1.47
	2	8.20	7.40	5.43	2.09	1.30
	3	14.90	6.25	5.21	1.95	1.26
	4	14.25	5.81	4.65	1.96	1.03
	5			4.90		1.20
	6					0.98
	Average	11.66	6.49	5.04	1.97	1.21

The most resistant strain of *P. fragi* was Strain B. The two strains of *P. viscosa* were eliminated when experiments showed that the available strains of *P. fragi* were considerably more heat-resistant.

Developing thermal resistance data. The *D* value is the reciprocal of the slope of a survivor curve, the time in minutes for a survivor curve to pass over one log cycle (10). The *D* value was calculated by the method of least squares from the logarithms of the numbers of survivors for each trial with each test culture at each temperature. The *D* values are in Table 2. All results obtained in a heat-treatment trial were used in calculating the *D* value, though there was a lag in the initial destruction of some cultures. Selecting results so as to eliminate lag from the survivor curves did not give appreciably different *D* values.

The *z* value is the reciprocal of the slope of a thermal resistance curve, the number of degrees F. required for a thermal resistance curve to pass over one log cycle (10). For each test culture the *z* value and the equation for the thermal resistance curve were calculated by the method of least squares from the logarithms of the average *D* values in Table 2. The thermal resistance curves and a summary of certain important values calculated for the selected test cultures are in Figure 1 and Table 3. The calculated *D* values used to position the thermal resistance curves (Figure 1) were calculated with the regression equations.

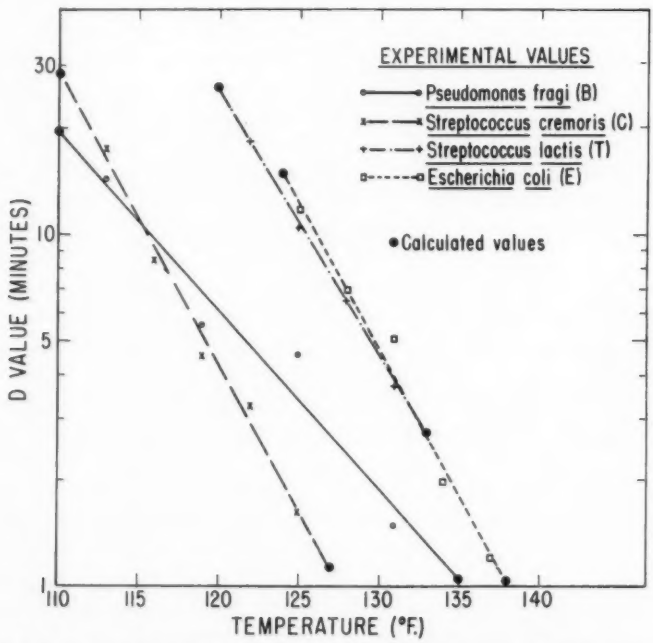

FIG. 1. Thermal resistance curves for the selected strains of bacteria.

TABLE 3
Important values calculated for the selected strains of bacteria

Strain	*D* value at 130° F.	*z* value	Regression equation
	(min.)	*(° F.)*	
E. coli (E)	4.73	12.07	$\log D = 11.4464 - 0.08286T$
P. fragi (B)	1.88	19.71	$\log D = 6.8699 - 0.05073T$
S. cremoris (C)	0.64	12.12	$\log D = 10.5264 - 0.08248T$
S. lactis (T)	4.57	13.26	$\log D = 10.4635 - 0.07541T$

Determining lethality of cooking procedures. The procedures used in cooking Cottage cheese curd in seven California plants were evaluated as to lethality for each test culture. An attempt was made to select plants representing the maximum variations encountered in California. Additionally, a hypothetical procedure, with 121° F. as maximum temperature and a holding time of 20 min. at 120° F. or above, was used to represent a cooking procedure that has been widely used, according to Angevine (1).

Four to six vats of normal cheese curd were selected in each of the seven plants. Temperature of the whey was measured at intervals of 5 or 10 min., to enable plotting an average time-temperature curve for the cooking procedure. Figure 2 gives curves for the procedures used in these plants and for the hypothetical procedure. Large curd (L) differed distinctly from small curd (S) in cooking procedure in only two plants.

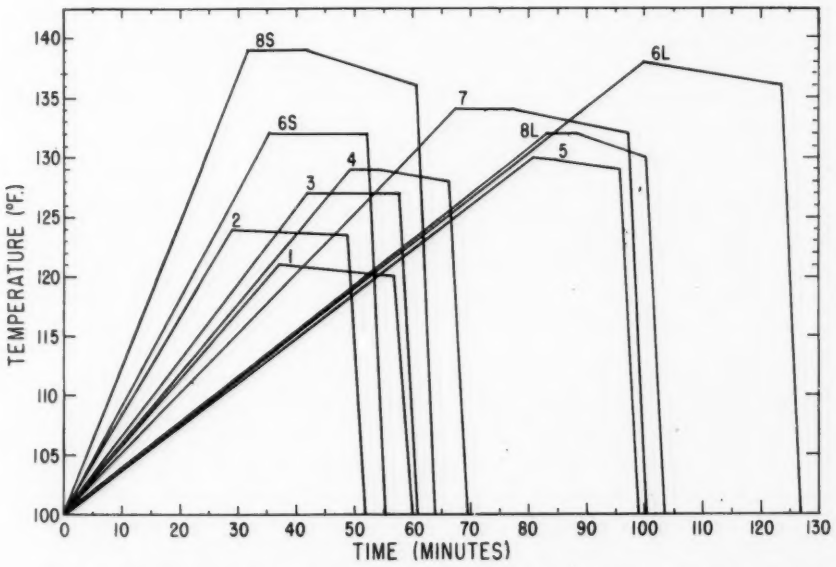

FIG. 2. Heat-treatment procedures used for cooking Cottage cheese curd in seven California plants. Procedure No. 1 is hypothetical, representing a widely used Cottage cheese cooking procedure.

The heat treatment above 100° F. involved in each Cottage cheese cooking procedure was evaluated as to lethality for each test culture in terms of minutes at 130° F., by a method similar to that applied to pasteurization by Burton (2). Results are in Table 4. Temperatures (at intervals of not greater than 3° F.)

TABLE 4

Lethalities of Cottage cheese cooking procedures expressed as minutes at 130° F. for each selected strain of bacteria

Procedure [a]	*S. cremoris* (C)	*S. lactis* (T)	*E. coli* (E)	*P. fragi* (B)
1	5.3	6.3	5.2	12.3
2	8.6	9.5	7.9	15.2
3	16.2	15.5	14.0	20.6
4	22.4	22.8	20.7	26.0
5	29.4	30.5	28.2	37.9
6S	35.3	35.0	34.5	35.4
6L	164.4	146.3	154.2	114.5
7	84.2	78.9	78.4	73.4
8S	163.7	146.6	155.6	98.2
8L	43.4	43.9	42.2	48.5

[a] S = small curd; L = large curd.

and corresponding times were taken from the curves shown in Figure 2. Lethal rate values (reciprocals of *D* values) for the temperatures of a heat treatment were calculated with the regression equation for the culture concerned. The lethal rate values were plotted against corresponding times, and the area under the curve was determined. The area formed is a measure of the lethality of the heat treatment for the test culture and can be used for comparative purposes. The area was evaluated in terms of minutes at 130° F., as follows: The lethal rate value for 130° F. was plotted on the same graph, and the area formed by a 1-min. holding time was determined. The area for the heat-treatment curve was divided by the area for the 1-min. holding time at 130° F.

DISCUSSION

The development of defects in Cottage cheese obviously is influenced by factors other than the specific microorganisms present. But the need for minimizing bacteria capable of producing spoilage has not been eliminated. This report, therefore, gives data that define the value of various cooking procedures in terms of destruction of selected psychrophiles, coliforms, and lactic streptococci.

Since destruction of a microorganism in unlimited amount of product can not be complete, conclusions on the probability that a microorganism will survive a given heat treatment must necessarily involve certain arbitrary values (4, 5). It is generally agreed that proper pasteurization of milk destroys psychrophilic bacteria known to produce defects in Cottage cheese, and coliform bacteria. Chaudhary *et al.* (3) found that selected species of *Pseudomonas* did not multiply during Cottage cheese setting and could not be isolated from the resulting cheese, cooked with 130° F. as the maximum temperature. A plate count of 10^4 per gram of psychrophiles or coliforms for Cottage cheese curd before cooking, then, would reflect gross contamination after pasteurization.

But with this hypothetical initial population of *P. fragi,* and with one organism per 200 lb. of cheese assumed as the number that will be tolerated, 9 log cycles of destruction (i.e., destruction to 10^{-9} of the initial population) must be accomplished in the cooking process (4). This requires 16.9 min. at 130° F. With 1 per 10 g. set as the maximum number of *E. coli* that will be tolerated in cheese, 5 log cycles of destruction, requiring 23.7 min. at 130° F., must be accomplished in cooking. Uncooked Cottage cheese curd normally contains about 10^{10} lactic streptococci per gram. If the number that will be tolerated in Cottage cheese curd after cooking is one organism per 2 lb., the cooking process must bring about 13 log cycles of destruction. At 130° F., 13 log cycles of destruction require 8.32 min. for *S. cremoris* (C) and 59.4 min. for *S. lactis* (T).

Comparison of the above figures to those in Table 4 shows that: (a) The heat treatments of Procedures 1 and 2 are insufficient to prevent the development of *P. fragi* (B). (b) Four procedures are unsatisfactory with regard to *E. coli* (E). (c) Only one procedure is unsatisfactory with *S. cremoris* (C) used as cheese culture, but seven of the ten procedures are unsatisfactory with *S. lactis* (T).

Cooking procedures without a maximum temperature of at least 130° F. and a holding time of at least 18 min. at the maximum temperature are unsatisfactory unless the rate of heating is very slow, as in procedures 5, 6L, and 8L (Figure 2). A procedure with the rapid heating of procedure 8S, a maximum temperature of 130° F., and a holding time of 18 min. at 130° F. would be satisfactory with regard to all of the selected cultures except *S. lactis* (T). The lethality for *E. coli* (E), expressed as minutes at 130° F., would be 23.9. Of the organisms studied, *S. lactis* is most heat-resistant and considered of least importance, since the author has rarely found this species in commercial Cottage cheese cultures.

Present procedures for cooking Cottage cheese curd vary considerably in actual heat treatment (Figure 2; Table 4). Some manufacturers may use procedures that are less effective in destroying microorganisms than the hypothetical procedure included in this study for comparison. The variations encountered make it obvious that manufacturers can, by adjusting certain of the several factors that influence the expulsion of whey by heating, alter heat treatment considerably without harming the physical properties of the resulting Cottage cheese.

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EFFECT OF pH AND BRINE CONCENTRATION ON THE THERMAL RESISTANCE OF PA 3679 IN A PROCESSED CHEESE SPREAD [1, 2]

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SUMMARY

Tests conducted on a processed cheese spread inoculated with PA (putrefactive anaerobe) 3679 and packaged in TDT (thermal death time) cans indicated that only a small amount of heat would prevent gas production as measured by can expansion, whereas a considerably greater amount of heat would kill the spores. The average D_{250} values of PA 3679 in cheese spread at pH 5.50, 6.25, and 7.00 were 0.67, 1.01, and 1.21 min., respectively, with a z value of 18° F.

The results suggest that a surviving spore population in the range of 10^3 to 10^5 per gram is necessary for gas production by this organism in the processed cheese spread tested. As the processing temperature increased, the calculated number of spores necessary to initiate gas production decreased.

The thermal resistance of a spore suspension of PA 3679 in neutral phosphate buffer was equivalent to a D_{250} value of 0.98 with a z value of 17.5° F., as measured with the thermoresistometer technique and subsequent subculturing in liver infusion broth.

Heat treatment may be used to render processed cheese spreads virtually free of spoilage organisms without undue damage to the product (9, 10). However, under commercial processing procedures there have been instances of gas production attributable to spore-forming anaerobes in the cheese. The objective of this study was to determine the thermal resistance of PA (putrefactive anaerobe) 3679 in a processed cheese spread under varying conditions of pH and brine concentration. The problem was studied in two parts: (a) the effect of pH and brine concentration on the thermal resistance of the spoilage organism; and (b) the effect of pH and brine concentration on the heat treatment required to prevent gas production by the spoilage organism.

EXPERIMENTAL PROCEDURE

The thermal resistance of a spore suspension of PA 3679 prepared by Jaynes *et al.* (7) was determined using a thermoresistometer which has negligible heating and cooling lags (11). Heat resistance was evaluated at temperatures of 240, 245, 250, 255, and 260° F. At each of these temperatures ten replicate

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[2] This manuscript describes a portion of the research contained in a thesis prepared by the senior author in partial fulfillment of the requirements for the Doctor of Philosophy degree at Michigan State University.

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samples were tested at eight to ten different time intervals. After the heat treatment the samples were incubated at 98.6° F. in stratified tubes of liver infusion broth (7). Both positive and negative growth occurred in the series of tubes in at least two of the time-temperature combinations tested at each of the above-designated temperatures, enabling use of the Schmidt (14) method to evaluate the data.

All tests to determine the effect of pH and brine concentration on the thermal resistance and the heat treatment required to prevent gas production of PA 3679 were made in TDT (thermal death time) cans (208 × 006). The TDT cans were filled with 19 g. of inoculated standardized processed cheese spread substrate prepared by Jaynes *et al.* (7). The substrate contained an amount of spore suspension calculated to give 3.3×10^6 spores per can or 1.74×10^5 spores per gram, salt to give the desired brine concentration, and either 6.0 *N* NaOH or HCl as necessary to give the desired pH. The TDT cans of inoculated cheese spread were allowed to equilibrate at 56° F. before being heated in a flat position in the miniature retorts. All heating times were corrected for heating and cooling lags (8).

Tests to determine thermal resistance. Thermal resistance studies of PA 3679 were made on inoculated batches of standardized processed cheese spread at pH levels of 5.50, 6.25, and 7.00 and at brine concentrations of 2.0 and 4.4% for each of the three pH levels. Processing temperatures of 225, 230, 235, 240, and 245° F. were used. TDT cans of cheese prepared and processed as described above were incubated overnight at 98.6° F., then approximately 0.01 g. of product from each can was removed with a loop and subcultured in stratified liver infusion broth. The 0.01-g. sample was obtained by stabbing the central area of the aseptically opened TDT can of product in three different places with a calibrated loop. After the third stab, the loop of product was introduced into the stratified tube of subculture medium. All tubes were incubated at least 3 mo. at 98.6° F. Five replicate TDT cans were used at each time-temperature combination and one tube was subcultured from each can. The remaining portion of cheese was subjectively evaluated for color. The D values were calculated for the data of each temperature and the F values of the contents of the TDT cans were calculated from the D values, using the methods of Schmidt (14).

Tests to determine heat treatment required to prevent gas production. Studies on the gas production by PA 3679, as evidenced by expansion of TDT cans, were made on batches of standardized cheese at pH levels of 6.0 and 7.0, brine concentrations of 1.6 and 2.4%, and processing temperatures of 220, 225, 230, and 235° F. The thickness of each of ten replicate TDT cans incubated at 98.6° F. at each time, temperature, pH, and salt combination were measured, using a dial micrometer technique, previously described by Jaynes *et al.* (7). The cans were measured daily for the first three days after heat processing, then monthly for 5 mo. All measurements for thickness of cans were made at a constant temperature of 98.6° F. Results in terms of positive can expansion and no can expansion were plotted on semilogarithmic paper and a straight line drawn above all positive values.

RESULTS AND DISCUSSION

Effect of pH and brine concentration on thermal resistance. The thermal resistance of the spore suspension of PA 3679 heated in M/15 phosphate buffer and subcultured in liver infusion broth was normal for this organism under these conditions; the D_{250} was 0.98 min. and the z was 17.5° F.

Results of the test to determine the thermal resistance of PA 3679 in cheese spread at pH levels of 5.50, 6.25, and 7.0, with brine concentrations of 2.0 and 4.4%, are presented in Table 1 and shown graphically in Figure 1. F values were calculated using Schmidt's (14) equation:

$$F = D \ (\log \ A + 2)$$

Since the initial number of organisms (A) in the can was 3.3×10^6, then $F = 8.52 \ D$. The D_{235} values decreased with reductions in the pH, but were not affected by variations in brine concentration. The thermal resistance of

TABLE 1

Effect of pH and brine concentration on D_{235}, F_{235}, and z values for TDT cans of cheese spread containing 3.3×10^6 spores of PA 3679 per can (0.01 g. from each can subcultured in liver broth and incubated at 98.6° F. for 3 mo. or more)

pH	Brine concentration (per cent)					
	2.0			4.4		
	D_{235} (min.)	F_{235} (min.)	z (° F.)	D_{235} (min.)	F_{235} (min.)	z (° F.)
5.50	4.6	39.2	18.25	4.5	38.3	19.25
6.25	6.8	58.0	18.75	7.0	59.6	18.25
7.00	8.4	71.6	17.75	8.1	69.0	17.00

the spores is significantly greater at pH 7.0 and 6.25 than at pH 5.5, as determined from the 95% confidence limits. Differences in the thermal resistance of the spores between pH 6.25 and 7.0 are not statistically significant. The decrease in the thermal resistance of the spores at reduced pH values observed in this work verified the findings of other investigators (2-4, 13, 15).

Thermal resistance curves vary with the methodology used in obtaining them. The error involved in removing 0.01 g. of sample from each TDT can for subculturing is estimated to be about plus or minus 20%. Since logarithms are used in the calculations, there would be practically no change in the calculated D values unless the subcultured samples were either at least ten times greater or ten times smaller than 0.01 g. In addition to the error in sample weight, there are inherent errors attributable to nonuniform distribution of the spores and to determination of the most probable numbers.

Failure of variations in brine concentration to affect the thermal resistance of the spores substantiated the results of Yesair and Cameron (18), who reported that the thermal resistance of spores of *Clostridium botulinum* was unaffected by different concentrations of salt when the samples were subcultured in an optimum growth medium. Salt did not show any protective influence in this study, which is contrary to results reported by some other workers (5, 16, 17).

The relative position of the color curve and the F curves in Figure 1 indi-

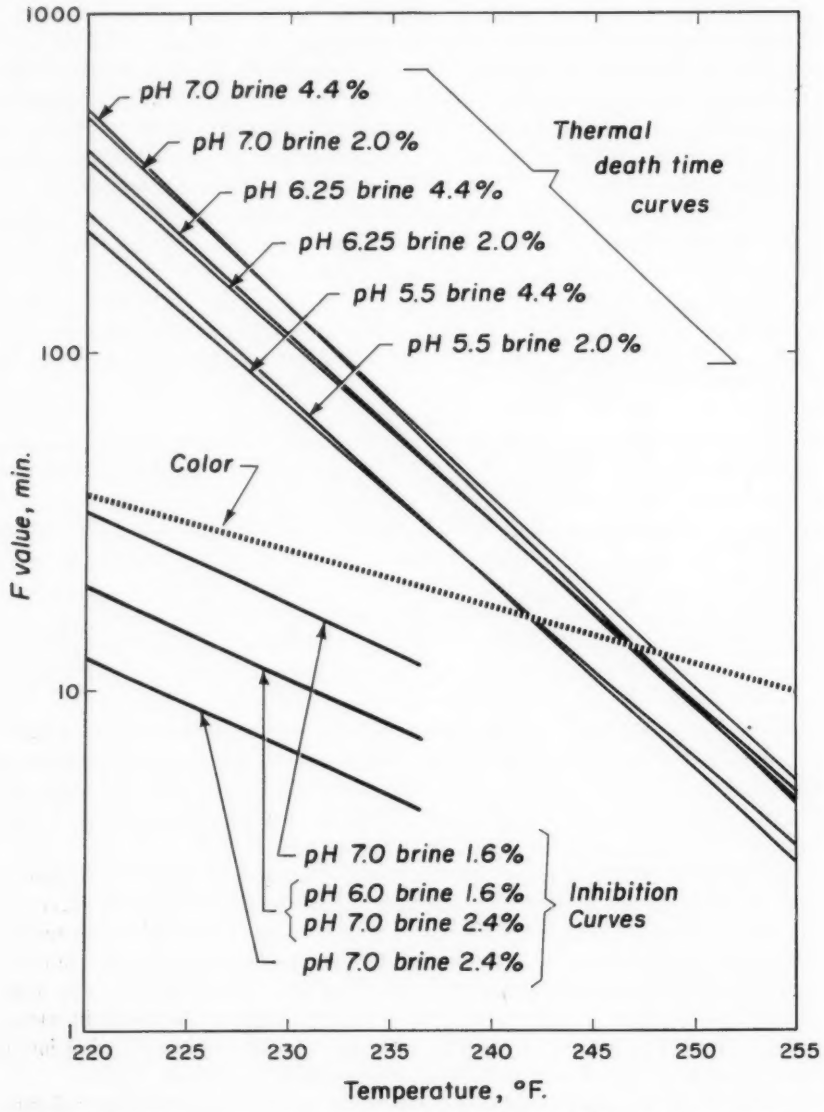

FIG. 1. Graphical summary showing thermal death time, inhibition, and color curves.

cate that thermal processes at higher temperatures for shorter times are preferable to low-temperature long-time processes for producing a commercially sterile product with an acceptable color. Under conditions of this study, minimum temperatures that could be used to commercially sterilize TDT cans

(208 × 006) of processed cheese spread at pH 5.50, 6.25, and 7.00, and maintain an acceptable color, were 242, 247, and 248° F., respectively. This points out the necessity for using high temperatures for short times to obtain a commercially sterile processed cheese spread with an acceptable color. These conclusions agree with Ball (1), who pointed out that, in proportion to their destructive effect on bacteria, high temperatures have less effect on quality impairment than low temperatures. Under conditions of these tests, an F_{250} of approximately 10 min. was required for commercial sterility at a pH of 6.25 or 7.0.

Effect of pH, brine concentration, and the thermal process on the number of spores necessary to cause expansion of cans. Data presented in Table 2 and

TABLE 2

Effect of heat treatment (U) at different retort temperatures on the expansion of TDT-cans of cheese spread at several pH and brine concentrations (3.3×10^6 spores of PA 3679 per can and incubated at 98.6° F. for 5 mo.)

Retort temperature							
220° F.		225° F.		230° F.		235° F.	
U (min.)	Expansion (in.)	U (min.)	Expansion (in.)	U (min.)	Expansion (in.)	U (min.)	Expansion (in.)
pH 6.0 and a brine concentration of 1.6%							
7.6	0.112	5.4	0.100	4.0	0.115	2.9	0.107
12.5	0.095	9.0	0.089	6.6	0.094	4.8	0.098
20.0	0.022	14.5	0.025	10.5	0.051	7.8	0.057
33.0	0.000	24.0	0.000	18.0	0.000	13.0	0.000
pH 6.0 and a brine concentration of 2.4%							
4.6	0.063	3.4	0.061	2.5	0.048	1.8	0.041
7.6	0.031	5.4	0.040	4.0	0.039	2.9	0.029
12.5	0.017	9.0	0.017	6.6	0.015	4.8	0.018
20.0	0.000	14.5	0.000	10.5	0.000	7.8	0.000
pH 7.0 and a brine concentration of 1.6%							
12.5	0.094	9.0	0.103	6.6	0.108	4.8	0.104
20.0	0.073	14.5	0.067	10.5	0.087	7.8	0.087
33.0	0.030	24.0	0.015	18.0	0.036	13.0	0.041
55.0	0.000	40.0	0.000	29.0	0.000	21.0	0.000
pH 7.0 and a brine concentration of 2.4%							
7.6	0.063	5.4	0.057	4.0	0.063	2.9	0.061
12.5	0.045	9.0	0.044	6.6	0.047	4.8	0.044
20.0	0.026	14.5	0.027	10.5	0.017	7.8	0.026
33.0	0.000	24.0	0.000	18.0	0.000	13.0	0.000

illustrated in Figure 1 show that the F values derived from the data on can expansion are less, and the z values greater, than the F and z values for spore destruction. These results agree generally with those of Reynolds (12), who observed z values ranging from 14.5 to 27.5° F. for PA 3679 when the curves were based on the results of incubation tests of the food substrate. The results also agree with Townsend *et al.* (16), to the extent that F values are lower and z values are different when the spores are incubated in a food substrate slightly inhibitory to growth, as compared to incubation of the spores in an optimum growth medium.

The theoretical number of viable spores remaining (B) in the cans of processed cheese spread showing expansion following heat treatment at the longest heating time, and no expansion at the shortest heating time, were calculated, using the thermal destruction data in Tables 1 and 2; these are tabulated in Table 3. Values were calculated using the equation

$$\log B = \log A - \frac{U}{D}$$

The value of A was 3.3×10^6, the initial number of spores per TDT can. Values of U are those given in Table 2 for the longest heating time at which expansion of cans occurred and the shortest heating time at which there was no expansion. D values were obtained from the data in Table 1. At pH 7.0, the two values were averaged to give a D_{235} of 8.25 min. At pH 6.0, it was necessary to interpolate between pH 5.5 and 6.25 to obtain the D value of 6.25 min. D values at 220, 225, and 230° F. were calculated using the D_{235} values and a z value of 18.2° F., which is the average of the z values of all the tests recorded in Table 2. The following equation was used

$$Dt = D_{235} \cdot 10^{\frac{235 - t}{z}}$$

Data in Table 3 reveal that a relatively large number of spores are necessary to initiate gas production in the inhibitory cheese substrate. It is of interest that apparently fewer spores were required to produce can expansion in processed cheese spread at pH 7.0 and a brine concentration of 1.6% than at pH 6 and a brine concentration of 2.4%; also, that the calculated number of spores necessary to initiate can expansion decreases with increases in the processing temperature. This would seem to indicate that some inhibitory factor is partially destroyed at the higher temperature or that some limiting nutrient or other stimulating factor becomes more available.

It should be pointed out that failure of an organism to produce gas does not necessarily mean that germination and multiplication were completely

TABLE 3

Calculated number of spores in cans showing expansion at longest heating time and no expansion at shortest heating time, as influenced by pH, brine concentration, and processing temperature

pH	Temperature (° F.)	No. of spores in thousands at brine concentration of: 1.6%		2.4%	
		No growth	Growth	No growth	Growth
6.0	220	533	1,094	1,094	1,656
	225	274	733	733	1,294
	230	98	425	425	910
	235	27	186	186	562
7.0	220	331	852	832	1,429
	225	141	497	497	1,052
	230	44	227	227	692
	235	9	88	88	373

inhibited. Greenberg, Silliker, and Fatta (6) point out that high brine levels make it possible for *Cl. botulinum* to produce toxin in canned meats with no organoleptic change or gas production. Because of the possible hazards, it is suggested that processes for cheese spreads be used that will insure the destruction rather than the inhibition of potential spoilage organisms.

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EFFECTS OF FEEDING LOW-LEVEL CHLORTETRACYCLINE TO A DAIRY HERD CONTINUOUSLY FOR ONE YEAR[1]

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SUMMARY

The effects of feeding chlortetracycline at the low level (0.1 mg. per pound of body weight, or 0.2 p.p.m.) were studied for a continuous period of 12 mo. on 34 pairs of Guernsey cows in one herd. Under the conditions of this experiment, the incidence of diseases such as foot rot, bloat, metritis, and milk fever was low in both treated and untreated groups. There was a moderate number of mastitis cases in both groups, with no significant difference between them. Differences found in the production of milk, fat, 4% FCM, 4% FCM-ME, fat percentage, persistency of lactation level, daily peak production, or average yield per month were statistically not significant. A comparison of the initial 15 cow pairs showed tendencies in milk, fat percentage, and 4% FCM production in favor of the chlortetracycline treatment, but differences were statistically significant only for fat and 4% FCM production. This experiment with lactating cows and a low-level oral dosage of chlortetracycline did not seem to add new support to a concept of direct metabolic action by chlortetracycline, on the basis of the criteria used for evaluation, even though there was a tendency for the chlortetracycline-fed cows to have an increased fat content in the milk.

The antibiotic activity of chlortetracycline against bacteria and protozoa is used in the treatment of cattle (18, 33). Chlortetracycline may be mixed into a dairy feed for this reason. Studies on the mode of action of chlortetracycline produced results, however, which are not fully explained by mere antibiotic effects upon gastro-intestinal microbial populations. The possibility of a direct metabolic effect of chlortetracycline is indicated by several publications dealing both with ruminants and with nonruminants. Growth response was obtained despite low disease levels (2, 15) or in the absence of chlortetracycline in the rumen when it had been injected instead of being fed (14, 37). Responses differed depending on sex, i.e., were found only in males (19, 29). Increased adrenal cortex function (31), inhibition of thyroid activity (6), and stimulation of the secretion of pituitary growth hormone (38) were noted. Changes in blood lipoid levels (19, 32), increased blood sugar levels (8, 22, 44), higher liver and muscle glycogen deposition (31), and increased incorporation of C^{14}-acetate, not attributable to intestinal microflora, into hepatic fatty acids, were reported. Decreased body temperature (40) and increased protein digestibility (30) were found. It has been proposed that chlortetracycline affects fat, protein, and energy metabolism (16, 23, 30, 42), that it acts at the cellular enzymatic level in the liver (1) or on other body tissues (12, 16) in addition to its antibiotic action. Work with animals, particularly at low disease levels or germ-free conditions, seems to support this concept (2, 15).

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Mixing of chlortetracycline into a dairy feed may, therefore, be justified, either for therapeutic or prophylactic reasons or for its direct metabolic action on the basis of evidence cited above. Such action in the case of dairy cows might be expected to manifest itself in terms of increased milk production or changes in milk composition. Earlier reports (3, 4, 21, 23, 35, 36, 39) concluded that there was no real advantage from feeding chlortetracycline on a routine basis to lactating cows. Other recent reports indicated increased milk and fat production due to chlortetracycline (5, 7, 11, 24) and oxytetracycline (27). Some of these reports are based on trials with a few cows, of only a few weeks' duration, or were conducted in the field with cooperating farms. This paper reports the results of feeding chlortetracycline continuously under controlled conditions to a herd of Guernsey cattle.

EXPERIMENTAL PROCEDURE

The registered Guernsey herd of the University of Delaware, with frequent cases of foot rot, mastitis, metritis, and pneumonia in previous years, seemed to be suitable (13, 18, 33) for a study of long-time effects of chlortetracycline upon dairy cows. However, a greatly reduced disease incidence was encountered in both groups during the period of the experiment.

Sixty-eight cows were used during the course of the trial. The 30 cows lactating at the beginning of the experiment were paired and started on the experiment. Twenty-two additional cows, ten of which were first-calf heifers, were placed on the experiment as they calved. Sixteen of the original 30 cows calved during the experiment and returned to the experiment as "new cows." In all the above, the animals were paired as closely as possible as to estimated producing ability, age, and stage of lactation. One animal from each pair was assigned on a random basis to the treatment groups.

During the month prior to the experiment, the daily production averages of the 30 original cows (Table 3) were in Group A (chlortetracycline): 17.6 lb. milk, 4.8% fat, 0.85 lb. fat, and 19.7 lb. 4% FCM (fat-corrected milk); in Group B (control): 17.0 lb. milk, 4.6% fat, 0.76 lb. fat, and 18.2 lb. 4% FCM (see Figure 1). These production averages were not statistically different ($P < 0.05$).

Average age of all cows when placed into the experiment was in Group A: 4.7, in Group B: 5.1 yr. The average number of all previous lactations was A: 1.9, B: 2.4. The cows in Group A weighed 960 lb., in B: 980 lb. in average. The fifteen original pairs at the start of the trial were 5.2 mo. in milk in Group A, 5.7 in Group B; their average length of gestation was 1.8 mo. in Group A and 2.4 in Group B. Group B contained a few older cows and was also slightly further along in lactation, which was considered in the analysis of the data (Table 3).

In order to avoid intentional or unintentional bias in the treatment effects through management, the designation of the experimental groups was by the letters A and B, so that the barn labor, including the herdsman, did not know the identity of the treatment groups. Each group received 65 g. of a feed sup-

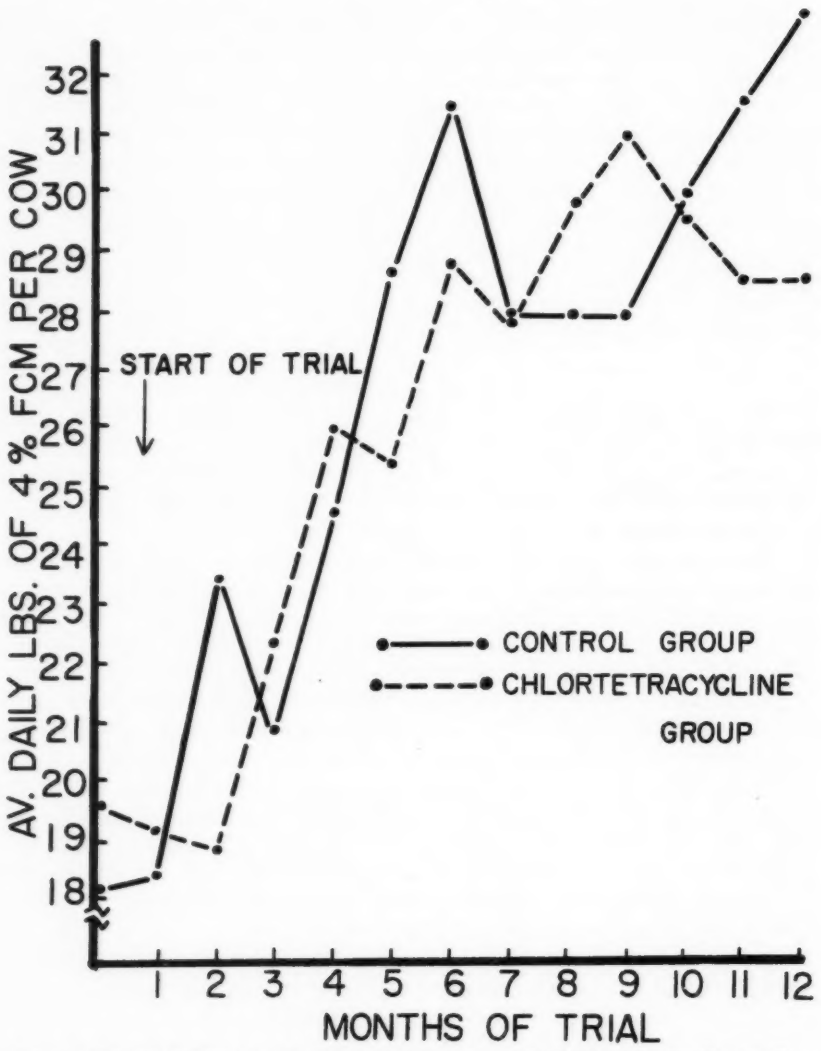

FIG. 1. Effect of feeding low-level chlortetracycline upon 4% FCM production by months.

plement, by volume (1 cup), per head per day, beginning December 1, 1958, and ending November 30, 1959.

Feed supplement A consisted of corn gluten feed with 1,538.5 p.p.m. or 100 mg. chlortetracycline[3] per 65 g. of feed (the daily dosage per cup for

[3]Chlortetracycline in the form of Aurofac 10, obtained through the courtesy of the American Cyanamid Company, New York, contained 10.0 g. chlortetracycline hydrochloride per pound.

each animal). This daily low-level administration provided each cow with 0.1 mg. of chlortetracycline per pound of body weight, or 0.2 p.p.m. per average body weight of 1,000 lb. This level was recommended by the manufacturer of chlortetracycline and had been reported to cause no detectable amounts of the antibiotic in milk of cows (43).

Feed supplement B for the control group, to provide a differently colored feed, consisted of alfalfa meal with 13% black strap molasses and contained no chlortetracycline. Supplements A and B were easily distinguished by their different colors, yellow vs. blackish-green. They were fed on top of the grain ration (grain : milk ratio = 1:3.5) during the morning feeding. In addition, the herd feed consisted of corn silage, alfalfa-grass hay, and grass pasture.

The two experimental groups were stabled separately in two 15-stanchion rows of the college Guernsey barn, where all the milking and feeding were carried out throughout the year. Milk production was recorded twice daily. Fat content was determined once monthly from composite samples of two milkings, using the Babcock method. The 4% FCM production was calculated by Gaines's formula: FCM = 0.4(lb. milk) + 15(lb. fat) (34).

Persistency of lactation was determined by three methods. Fischer (10), in a study of four different methods, found the $P_{2:1}$ Index the most accurate measure of lactation persistency. This $P_{2:1}$ Index, developed by Johansson in Sweden, expresses the second 100-day milk production in per cent of the first 100-day production. Lennon and Mixner (26) proposed a P index, which is calculated from the last six monthly test days of a 240-day lactation by regression. However, in order that all cow data may be used, persistency values were also calculated on the basis of milk flow decrease in pounds per cow per week (25).

Cases of infectious diseases and disorders were diagnosed and treated by the attending veterinarian of the college herds[4] and recorded daily.

Statistical analyses were made on the basis of paired observations (44) by Student's "t" test.

RESULTS AND DISCUSSION

At the end of the 12-mo. trial period, 34 pairs of Guernsey cows had been in the experiment. During this time, a total of 525 g. of chlortetracycline was consumed by Group A as a supplement to the regular feed.

Diseases and disorders. The incidence of diseases and disorders was reduced in both groups, compared with the previous year. A reorganization of the barn management, to insure proper care of the college herds, may have contributed to this result. Details about the occurrence of diseases are given in Table 1.

Twenty-one of the 68 cows had an outbreak of mastitis, with ten cows in the chlortetracycline and 11 cows in the control group. The total numbers of days-with-mastitis seem to be different at first. But the individual cow data showed that 97 days in the control group are due to the contribution of a single old cow with chronic mastitis that resisted repeated treatments by the veterinarian.

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TABLE 1

Incidence of diseases in a Guernsey herd during one year of low-level feeding of chlortetracycline

Item	Group A (Chlortetracycline) 34 cows	Group B (Control) 34 cows
Mastitis		
No. of cows	10	11
No. of cow-days	61	165[a]
No. of cow-days adjusted [a]	61	68
Metritis	1	....
Foot rot	1	1
Bloat	1	1
Abortion	1	....
Milk fever	2	....
Ketosis	....	1
Pneumonia	....	....
Diarrhea	....	....

[a] See text for explanation of this value.

In the subsequent lactation, this cow entered the chlortetracycline group without recurrence of mastitis and also continued this way after termination of the experiment. The 97 days with mastitis of this old cow should, therefore, be eliminated from calculations, leaving 61 cow-days with mastitis in Group A and 68 cow-days in Group B.

The occurrence of foot rot, bloat, and other diseases was low, and between both groups there was also no significant difference. Similar results have been reported before (4, 7, 35), but it is in contrast to some other reports (18, 24).

Milk production. Average daily milk production per cow in the two experimental groups is shown in Table 2. Differences between treatment and control

TABLE 2

Milk production of a Guernsey herd during one year of low-level feeding of chlortetracycline

Item per cow per day	Group A (Chlortetracycline) 34 cows	Group B (Control) 34 cows	Difference due to Treatment "A"	LSD [a] at $P < 0.05$
Milk *(lb.)*	22.8	23.3	−0.50 (N.S.)	1.77
Fat *(%)*	4.82	4.67	+0.15 (N.S.)	0.24
Fat *(lb.)*	1.06	1.08	−0.02 (N.S.)	0.08
4% FCM *(lb.)*	25.1	25.7	−0.60 (N.S.)	1.27
4% FCM—ME [b] *(lb.)*	27.1	27.2	−0.10	

[a] LSD = Least significant difference (44).
(N.S.) = Difference not statistically significant at $P < 0.05$.
[b] ME = Mature equivalent (20).

groups in milk, fat, fat percentage, 4% FCM, and 4% FCM on a mature-equivalent basis were not statistically significant, even though fat percentage had a tendency to be higher due to chlortetracycline feeding. Some authors published similar results (4, 11, 21, 23, 35), whereas others reported an increased milk production after chlortetracycline feeding (5, 7, 25).

The data in Table 2 may be separated into those of the original cows which started the experiment and those that entered the groups during the course of the year. The results of such a calculation are presented in Table 3. Milk pro-

TABLE 3

Milk production of the original 15 cow pairs prior to and during low-level feeding of chlortetracycline

Items per cow per day	Group A (Chlortetracycline) 15 cows	Group B (Control) 15 cows	Difference due to Treatment "A"	LSD [a] at $P < 0.05$
	Month prior to trial			
Lactation days	30	30	0	
Milk *(lb.)*	17.6	17.0	+0.60(N.S.)	2.38
Fat *(%)*	4.77	4.57	+0.20(N.S.)	0.49
Fat *(lb.)*	0.85	0.76	+0.09(N.S.)	0.14
4% FCM	19.7	18.2	+1.50(N.S.)	2.94
4% FCM—mature-equivalent basis	21.2	19.2	+2.00(N.S.)	3.20
	During trial—data on calendar basis [b]			
Lactation days	161	146	+15	
Milk *(lb.)*	14.5	13.4	+1.10(N.S.)	1.36
Fat *(%)*	5.25	4.69	+0.56*	0.43
Fat *(lb.)*	0.74	0.61	+0.13**	0.07
4% FCM *(lb.)*	17.0	14.7	+2.30**	1.39
	Data on equal lactation segment basis [c]			
Lactation days	147	147	0	
Milk *(lb.)*	14.5	13.9	+0.60(N.S.)	1.35
Fat *(%)*	5.25	4.94	+0.30(N.S.)	0.47
Fat *(lb.)*	0.75	0.68	+0.07(N.S.)	0.08
4% FCM	17.0	15.7	+1.30(N.S.)	1.63
	Data on mature-equivalent, equal lactation segment basis [d]			
Lactation days	147	147	0	
Milk *(lb.)*	15.6	14.6	+1.00(N.S.)	1.24
Fat *(%)*	5.25	4.95	+0.30(N.S.)	0.47
Fat *(lb.)*	0.81	0.71	+0.10*	0.09
4% FCM	18.5	16.6	+1.90*	1.82

[a] LSD = Least significant difference (44).
(N.S.) = Difference not statistically significant at $P < 0.05$.
* = Significant difference at $P < 0.05$.
** = Highly significant difference at $P < 0.01$.
[b] December 1, 1958, until end of each cow's lactation.
[c] Lactations of cows in Group A compared only with equal segments and length of lactations of control cows (Group B).
[d] Using DHIA age-correction factors (20).

duction within the original 15 cow pairs did not differ significantly when the data were calculated on a calendar basis (December 1, 1958, until the end of each cow's lactation). Milk fat percentage and total fat production was found to be different and, consequently, the difference in 4% FCM production was also highly significant. However, the original chlortetracycline group averaged 5.2 mo. in milk and 1.8 mo. in gestation, whereas the control group was 5.7 and 2.4 mo., respectively, at the initiation of the trial. Therefore, milk production data were calculated furthermore on an equal lactation segment basis, comparing the lactations of the treatment cows with corresponding stages and lengths of

lactation of the controls. As shown in Table 3, the differences between the two experimental groups are not statistically significant, using Student's t-test for paired observations (44).

However, the ages of Groups A and B in these original cow pairs averaged 4.8 and 5.2 yr., respectively. Results of calculations of the data on a mature-equivalent basis, using standard DHIA correction factors (20) and allowing for these age differences, are also shown in Table 3. While milk production and fat percentage were not significantly different ($P < 0.05$), fat and 4% FCM production differed significantly. These results are of interest, particularly after comparing them with pretrial production data, also contained in Table 3. Before the trial the two groups differed in none of the criteria. However, the fat percentage of the milk in Group A increased during the trial sufficiently, making comparisons on a calendar basis or on a mature-equivalent, equal lactation-segment basis, significantly different for fat and 4% FCM production, as far as these original 15 cow pairs were concerned.

Further evaluation of the production data of all 34 cow pairs throughout the 12-mo. study, however, seemed desirable.

Persistency. It has been reported that "in an analysis of the variation association with lactation milk production on an among-cows-in-lactation-in-breed basis, the effects associated with the correlation of maximum initial milk yield accounted for 65.6% of the total variation; whereas, the additional effects due to persistency contributed 8.5% to the total variation" (26). Low-level feeding of chlortetracycline has been reported to cause cows in one trial to be "more persistent in milk production than the control cows" (24).

The data in Table 3 suggest that—even though milk production was not significantly different—because means of the initial cows tended to be larger in the chlortetracycline group—we might be able to find differences in persistency of lactation. Among the initially and subsequently entering cows there were nine cow pairs with a lactation of at least 240 days, to make calculations of the Persistency Index according to Lennon and Mixner (26) possible. This method is based upon the regression of the third to the eighth monthly test day of 4% FCM production, rather than on total pounds of milk produced, as in the P_{2a} method of Johansson, based upon the second 100 days' total production in per cent of the first 100 days' production (10). Thirteen cow pairs qualified for the latter method. The resulting persistency values with both methods are shown in Table 4. The differences due to treatment were not statistically significant for either of the two methods of calculation. Comparison on an equal calving-month basis did not change these results. To calculate persistency values on all 34 cow pairs in the experiment, a persistency value in pounds of milk flow decrease per cow per week was also used (25). Table 4 contains the results of such calculations. A tendency may be seen for the chlortetracycline cows to be more persistent, but the difference was not statistically significant.

Peak milk production. The maximum daily milk yields were examined for all cows on trial on a 4% FCM basis. The average maximum daily yield for the chlortetracycline group of 34 cows was 31.30 lb. in contrast to 31.27 lb. for the

TABLE 4

Persistency of milk production during low-level feeding of chlortetracycline

Item per cow	Group A (Chlortetracycline)	Group B (Control)	Difference due to Treatment "A"	LSD [a] at $P < 0.05$
Persistency index [b] *(%)* (9 pairs)	93.77	93.82	−0.05 (N.S.)	4.66
$P_{2/3}$ index [c] *(%)* (13 pairs)	89.08	87.64	+1.44 (N.S.)	12.28
Rate of decline [d] *(lb.)* (34 pairs)	0.309	0.375	−0.066 (N.S.)	0.075

[a] LSD = Least significant difference (44).
(N.S.) = Difference not statistically significant at $P < 0.05$.
[b] Regression of third to eighth monthly test 4% FCM (26).
[c] Second 100 days' total milk production in per cent to first 100 days (10).
[d] Rate of decrease of milk flow in pounds per week (25).

control group of 34 cows. No effect of treatment could be deduced from these data.

Monthly comparison. It seemed, finally, to be of interest to follow mean group milk production month by month, to gain a complete picture of the events during the 12 mo. of trial. This is shown in Figure 1. During the month prior to the trial, the chlortetracycline group had a higher average daily 4% FCM production than the control group. This reversed itself during the next months in the experiment. Both groups showed a parallel, almost uniform, increase of production during the 12 mo. of trial, and this was maintained even during the hot summer and fall months. Variation of production did not show a continuous advantage for the feeding of chlortetracycline. Whereas in some months the chlortetracycline cows produced more milk than the controls, in other months the controls produced more than the treatment cows, but apparent differences were not statistically significant ($P < 0.05$). If only this graphic method, and maybe only part of the year would have been used for comparison, false conclusions could have been made.

CONCLUSIONS

The following conclusions may be drawn from this experiment of feeding chlortetracycline to lactating cows at the low level (0.1 g. daily per 1,000 lb. body weight):

(1) The incidence of bloat, foot rot, mastitis, metritis, and milk fever was not affected under our experimental conditions.

(2) Milk production when data of all 68 cows were analyzed was not increased by feeding chlortetracycline. Fat content tended to be higher, but was not significantly changed. The initial 30 cows, however, showed a significant increase in fat and 4% FCM production due to chlortetracycline feeding, when data were calculated on a mature-equivalent, equal lactation segment basis.

(3) Persistency of milk production level was not significantly affected.

(4) Peak daily milk yields were not increased.

(5) 4% FCM averages per group per month did not differ significantly.

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RELATIONSHIP OF CERTAIN PLANT POLYURONIDES TO INCIDENCE OF PASTURE BLOAT IN DAIRY CATTLE [1]

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SUMMARY

Acid-soluble pectic substances, polyuronides, and pentosans were measured in plants from Ladino clover–alfalfa grass pasture plots grazed at 4-wk. intervals by dairy heifers and steers. The highest incidence of bloat occurred when Ladino and alfalfa were found to be highest in acid-soluble pectic substances. Daily changes in the polyuronide content and pentosans were less marked and were not associated with bloat. In an experiment with finely ground alfalfa clippings, one-third of the uronic acid equivalent was digested within one-half hour and accounted for most of the carbon dioxide produced. In vivo studies of rumen digesta revealed rapid disappearance of polyuronides within the rumen. Other studies showed (a) that phosphorus content of Ladino clover was unrelated to bloat, (b) that water-to-plant dry matter ratios required to form a stable mass in vitro were relatively constant within species for alfalfa and Ladino clover and were unrelated to content of pectic substances, and (c) that feeding grain once daily changed the diurnal pattern of bloating. It was concluded that the polyuronides, precursors of rapid gas production in the rumen and including the acid-soluble pectins, are plant compounds contributing to pasture bloat.

Past experimentation indicates that pasture bloat in cattle is mostly caused by interaction of plant factors, rumen microbial activity, and the particular physiology of the affected animal. Recent progress in research bearing on various aspects of causes of bloat has been thoroughly reviewed by Cole and Boda (4). This study concerns certain plant factors only and was undertaken to determine if polyuronide-containing substances in legume plants were related to the onset of bloat in dairy cattle.

In vitro studies revealed that the plant substances responsible for initial rapid gas production were closely associated with the fibrous fraction of the plant. These substances were removed after digesting 4 to 10 hr. with rumen microorganisms or by extracting 12 hr. with hot water. Also, pectin increased gas production when added to alfalfa residues prepared by expressing the juice from the whole plant under pressure and washing with cold water. Pectin incubated with the enzyme pectinase produced a greater amount of gas, yielding 2.25 times as much gas as did washed fiber (7). According to the studies of Head (11), the predominating carbohydrates in stable rumen froth were those in pectins and hemicelluloses. On the basis of these results, and the physical properties of pectins, it was postulated that leguminous pasture plants contain pectic and hemicellulosic compounds apparently responsible for the rapid froth formation in the rumen.

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EXPERIMENTAL PROCEDURES

Basic experiments consisted of grazing heifers and steers in rotationally managed pasture plots. Initial ages of the animals ranged from 8 to 12 mo. They were observed at 8 A.M., 1 P.M., and 5 P.M. on each work day, for symptoms of bloat. Plant content was related to the incidence of bloat by random sampling of the grazed forage plants daily.

Experiment 1—1958. Four heifers and four fistulated steers were grazed in turn for 1 wk. in four three-fourths-acre plots that had a 4-wk. period of regrowth. Previous studies using young heifers indicated that bloat would occur in pastures so managed (6). Ladino clover was the principal forage accessible to the animals, but scattered clumps of alfalfa, timothy, and weeds also were present. Plot 4 had significant amounts of alfalfa. Two heifers and two steers were penned separately each morning at 9 A.M. and fed grain concentrate containing corn, oats, and soybean oil meal at the rate of 2 lb. per head.

The sampled forages were immediately macerated in a food chopper and partitioned for chemical analysis. Pectic substances and dry matter were determined on the fresh material, whereas phosphorus content was measured after drying. Newly cut samples of alfalfa and Ladino clover were subjected to the mass test (19) and were compared for ability to form stable foam in relation to incidence of bloat. Also, the amount of dry matter required for stable foam formation was determined in each type of plant. Observed cases of bloat were rated for severity on the basis of published criteria (13), to the extent that judgment of the observers would allow.

Once during each of three successive weeks, the pH of the ruminal contents of the fistulated steers was determined in the top layer of rumen digesta, the lower layer of rumen digesta, and the reticular contents, using a Beckman Model G pH meter. pH in the various areas of the rumen was determined three times during the day, directly after removal of the digesta through the fistula. The steers were permitted to graze freely during the intervening periods.

Experiment 2—1959. Eight cattle were observed in four plots similar to those described in Experiment 1. A new plot with 4-wk. regrowth was used each week. Except for the first weekly period, when a pure stand of alfalfa was used, the botanical composition of pasture plots was estimated to consist of approximately equal amounts of timothy and alfalfa, with some weeds present. Ladino clover, the principal legume seeded, was winter-killed. No grain was fed during the course of the experiment. Because of dry weather supplementary pasture was used during two of the weekly periods. Observations were not made on the supplementary pastures. Daily observations for bloat and forage sampling for chemical analyses were carried out as described in Experiment 1. In addition to analyses for pectic substances and dry matter, plant polyuronides were determined daily as the uronic acid equivalent. Phosphorus determinations were discontinued.

As an additional phase of experimentation, four in vitro trials were carried out to ascertain the rate at which leguminous polyuronides were digested. One gram of dried, finely ground alfalfa containing 182 mg. of uronic acid equiva-

lent per gram was digested with 20 ml. of fresh strained rumen contents buffered with 20 ml. of one-fifteenth molar phosphate solution (8.1 g. of KH_2PO_4 and 10.1 g. of Na_2HPO_4 per liter). Digestion for times ranging between ½ and 6 hr. was carried out at 38.6° C. Nitrogen gas was bubbled through the digestion flasks for purposes of mixing and removing the fermentation gases. The disappearance of uronic acid and production of carbon dioxide were measured.

Experiment 3—1960. Eight heifers were grazed rotationally in eight one-half-acre pasture plots for three or four days. The heifers were changed on Mondays and Thursdays to succeeding plots having 4-wk. regrowth of forage. This afforded more access to ungrazed forage than in previous experiments. Ladino clover provided the major portion of the pasture. Remaining forages were timothy and alfalfa which, varying among the plots, were estimated on the basis of weekly observations to range between none and 50% of the forage present. Daily observations of the animals for symptoms of bloat were made as in previous experiments. Grain (corn, oats, and minerals) was fed once daily, in the evening, at the rate of 2 lb. per head. In addition to the pectic substance, uronic acid, and dry matter analyses, the pentosan content of each day's forage sample was determined on the dry material.

In conjunction with the studies on plant chemistry, diurnal changes in the uronic acid level of rumen contents were measured in (a) two fistulated steers after 2 hr. of morning grazing in Ladino clover pasture plots, (b) a fistulated steer after introducing 15 lb. of chopped alfalfa through the fistula, and (c) a bloated heifer taken from the pasture plots. In (c), rumen contents were obtained by stomach tube every ½ hour for 6 hr. Symptoms of bloat subsided during the first ½ hr. Contents from four areas of the rumen of the two fistulated steers were removed approximately every ½-hr. by forceps, and composited for analyses.

Analytical procedures. The acid-soluble pectic substances were determined on each of the daily samples, since it was thought they would be the most soluble pectic compounds in the rumen. Kertesz' procedure (14) was used, with some modifications. The acid-extracted pectic substances obtained are principally pectic and pectinic acid.

Plant samples were macerated in a household-type food grinder and 25 g. of each placed in a Weihe-Phillips apparatus and extracted five times with successive portions of 0.05 *N* hydrochloric acid at 80° C., using enough acid to cover the top of the plant material. Thirty minutes were allowed for each extraction. Enough concentrated hydrochloric acid was added to the total amount of extract obtained to render it 0.15 *N*. After mixing, 95% ethanol was introduced dropwise with constant stirring until a 2:1 mixture of ethanol and extract was obtained. The ethanol precipitates were allowed to stand 1 hr., then the precipitated pectic substances separated from the supernatant by centrifugation. The supernatant was then washed with a 2:1 mixture of ethanol and 0.15 *N* hydrochloric acid solution, dissolved in 10 ml. of warm water, and dried by lyophilization.

TABLE 1

Relationship of acid-soluble pectic substances in pasture plants to the incidence of bloat

Date	Plot No.	Ladino	Alfalfa	Grass	Average [a]	Bloat cases [b]
		(%)				
1958						
7-8 to 7-11	2	1.78	.....	.....	1.78	0.500
7-14 to 7-18	3	1.31	.....	.....	1.31	0.000
7-21 to 7-23	4	1.88	.....	.....	1.88	0.000
7-25 to 7-31	1	1.60	.....	.....	1.60	0.000
8-1 to 8-7	2	2.26	.....	.....	2.26	0.200
8-8 to 8-14	3	1.76	.....	.....	1.76	0.667
8-18 to 8-22	4	3.71	5.23	.....	4.47	1.800
8-25 to 8-29	1	4.09	4.39	.....	4.24	5.000
9-2 to 9-4	2	3.86	.....	.....	3.86	4.667
9-5 to 9-10	3	4.22	6.78	.....	5.50	3.800
1959						
6-22 to 6-26	A	.....	2.41	.....	2.41	0.200
6-29 to 7-3	4	.....	1.61	0.29	0.85	0.000
7-6 to 7-9	1	.....	2.84	0.12	1.48	0.250
7-13 to 7-15	2	.....	4.47	0.21	2.34	0.000
7-16 to 7-17	3	.....	3.79	0.20	2.00	0.000
8-3 to 8-7	4	.....	5.65	0.26	2.90	0.000
8-10 to 8-14	1	.....	7.46	0.38	3.92	0.000
8-17 to 8-21	2	.....	4.58	0.29	2.49	0.000
8-24 to 8-27	3	.....	2.85	0.12	1.49	0.000
9-7 to 9-10	1	3.71	4.02	0.21	2.56	0.200
1960						
6-13 to 6-15	1	2.40	.....	0.31	1.36	0.000
6-16 to 6-17	2	1.19	.....	0.48	0.84	0.000
6-20 to 6-22	3	2.00	.....	0.81	1.40	0.000
6-23 to 6-24	4	1.29	.....	0.56	0.93	0.000
6-27 to 6-29	5	2.34	3.20	0.83	2.13	0.000
6-30 to 7-1	6	2.75	4.42	1.13	2.77	2.250
7-5 to 7-7	7	2.80	5.56	.....	4.18	1.000
7-8 to 7-9	8	5.39	7.77	.....	6.58	1.500
7-11 to 7-13	1	2.31	.....	.....	2.31	1.667
7-14 to 7-15	2	2.61	.....	.....	2.61	3.250
7-18 to 7-20	3	1.25	3.03	.....	2.14	0.333
7-21 to 7-22	4	1.45	2.59	.....	2.02	0.000
7-25 to 7-27	5	1.38	2.84	.....	2.11	0.000
7-28 to 7-29	6	0.89	2.13	.....	1.01	0.000
8-1 to 8-3	7	1.83	2.48	.....	2.16	0.000
8-4 to 8-5	8	1.29	1.66	.....	1.47	0.000
8-8 to 8-10	1	3.10	.....	.....	3.10	1.000
8-11 to 8-12	2	2.18	.....	.....	2.18	0.000
8-15 to 8-17	3	2.47	2.08	.....	2.28	0.667
8-18 to 8-19	4	1.43	2.61	.....	2.02	0.000
8-22 to 8-26	5-6	1.90	2.87	.....	2.39	0.000
8-31 to 9-2	7-8	1.62	1.91	.....	1.77	0.000
9-8 to 9-12	1-2	2.02	2.79	.....	2.40	0.400
Av. bloat score		1.80	1.76		1.23	
Cow days		952	832		576	
Correlations [c]		0.70	0.65	N.S.	0.65	

[a] Average content for plants accessible, regardless of the amount of each actually eaten by the cattle.

[b] Number of bloat cases observed in eight cattle per day.

[c] Correlations highly significant; $P < 0.001$. Correlation for alfalfa involved only those values for plots wherein grass was not accessible. Complete data used for Ladino and average.

The procedure of McCready *et al.* (16) was used for estimating uronic acid, in which the galacturonic acid equivalent was determined by decarboxylating 10 g. of freshly macerated plant material with 19% hydrochloric acid. Pentosans

were determined by extracting finely ground plant material for 10 min. with 20% perchloric acid (17) and using the colorimetric procedures outlined by Brown (3). Briggs's colorimetric method (2) was used for phosphorus determination, and dry matter was obtained by oven drying overnight at 60° C.

RESULTS

Bloat incidence is summarized by grazing periods for the three summers' experimental periods, Table 1. Acid-soluble pectic substances in Ladino clover, alfalfa, and the pasture grasses are presented also, along with incidence data, as daily averages for the respective grazing periods (Table 1). Bloat was periodic; the greatest number of cases was observed during late August and early September of 1958. There was a high correlation between bloat incidence and content of acid-soluble pectic substances in the Ladino clover. Three animals were seriously affected and given relief by passing a stomach tube.

In 1959, only three cases of bloat were observed throughout the entire season. This was because timothy and orchardgrass were accessible during all grazing periods; often these grasses provided more than half of the available pasture plants. However, in 1960, Ladino clover was again the principal pasture plant. The greatest number of cases was observed during the 2 wk. between July 1 and 15. This also covered the grazing periods when the average per cent pectic substances was highest, but showed less relationship than the correlation coefficient obtained in 1958.

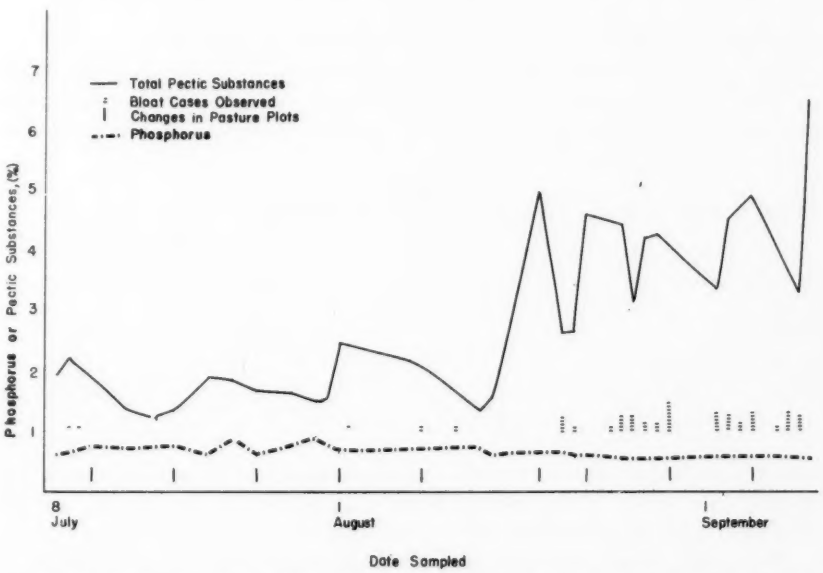

FIG. 1. Daily changes in acid-soluble pectic substances and total phosphorus content of Ladino clover.

Daily changes in the pectic substances of Ladino clover observed in 1958 are shown graphically, along with the average phosphoric content in the dry matter, Figure 1. These results vary widely with grazing dates; thus offer a possible explanation for the periodicity of bloat in cattle grazed continuously on the same type of forage. Changes in phosphorus content of the plant were not related to the onset of bloat.

Grain feeding altered the pattern of bloating. For example, the numbers of cases observed were 13, 5, and 13, respectively, for 8 A.M., 1 P.M., and 5 P.M. in the animals fed grain at 9 A.M. In contrast, in the group without grain, the numbers were 14, 14, and 19 for 8 A.M., 1 P.M., and 5 P.M., respectively. In connection with these studies, rumen pH values were obtained with four steers. Grain feeding resulted in higher acidity in most samples obtained from various areas of the rumen, Table 2. However, no relationship was found between the

TABLE 2

Range in pH of digesta from various areas of the rumen and reticulum of fistulated steers

Mean sampling time	Top of rumen		Bottom of rumen		Reticulum	
	Grain [a]	No grain	Grain [a]	No grain	Grain [a]	No grain
10:00 A.M.	5.21-6.00	5.72-6.32	5.20-5.90	5.82-6.29	5.77-6.22	6.45-6.56
11:20 A.M.	5.49-6.11	5.39-6.32	5.52-5.94	5.72-6.22	5.85-6.56	5.79-6.68
1:30 P.M.	5.45-6.40	5.71-6.36	5.49-6.15	6.08-6.61	5.74-6.52	6.18-6.58
3:00 P.M.	5.44-6.15	5.58-6.33	5.49-6.02	5.73-6.42	5.82-6.50	5.62-6.68
4:00 P.M.	5.75-5.99	5.75-5.97	5.75-5.85	5.82-6.01	5.87-6.32	6.19-6.31

[a] Fed 2 lb. of grain concentrate between the 10 A.M. and 11:20 A.M. samplings.

pH trends after grain feeding and the distribution of bloat cases.

The amount of pectic substances was not related to the amount of Ladino clover or alfalfa plant material required to form a stable mass when macerated with 100 ml. water in a Waring Blendor, Table 3. When converted to the dry

TABLE 3

Relationship of plant composition to the water-dry matter ratio of stable mass

	Pasture plant	
	Alfalfa	Ladino
No. of samples	5	5
Green material [a]	66	68
Dry matter *(%)*	20.1	12.2
Pectic substances *(%)*	4.9	3.6
Amount of water per gram of D.M. *(g.)*	11.5 ± 0.9	19.6 ± 1.6

[a] Green material required to form a stable froth.

matter basis, the quantity of plant tissue required to form a stable mass was remarkably constant among daily samples from the same species, but was less for alfalfa (11.5 g.) than for Ladino (19.6 g.).

The pectic substances were found in the leaves, leaf-stems, and stems of legumes, Table 4. The contents of acid-soluble pectic substances and polyuronides are presented also for various plants, Table 4. In all cases the legumes

TABLE 4

Acid-soluble pectic substances, polyuronides, and pentosans in dry matter of forages, stems, and leaves

Forage	No. of samples	Pectic substances	Poly-uronides	Pento-sans
		(%)		
Alfalfa	96	3.87	14.9	6.2
Ladino	96	2.41	14.5	4.7
Timothy	41	0.68	5.6	11.9
Orchardgrass	26	0.26	8.0	....
Bird's-foot Trefoil	4	2.45	11.8	....
Alfalfa leaves	6	1.97	15.6	....
Alfalfa stems	6	1.64	17.5	....
Ladino leaves	5	1.76	16.9	....
Ladino-leaf-stems	5	1.14	17.4	....

are highest in these substances. It is noteworthy that bird's-foot trefoil was as high in pectic substances as Ladino clover, but previous experimentation showed that trefoil would not support a stable mass in vitro (19). The grasses lowest in pectic substances were also low in polyuronides. Previous in vitro studies showed that grasses produced less gas per gram of material per unit time (19).

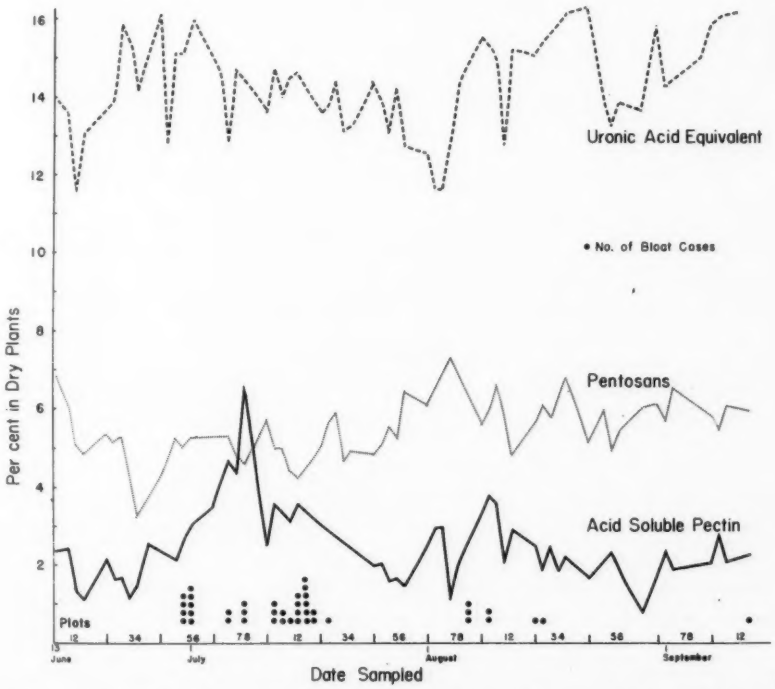

FIG. 2. The daily content of uronic acids (polyuronide equivalent), pentosans, and pectic substances for Ladino clover and alfalfa combined and averaged.

One aspect of the 1960 experiment was to relate pectic substances, total polyuronides, and total pentosans in the daily samples to bloat incidence. Results for both legumes combined are expressed graphically, Figure 2. Bloat was associated with increases in the content of pectic substances, but not related to changes in total polyuronides or pentosans. Polyuronides and pentosans were present in relatively large quantities, but remained reasonably constant through the grazing season.

The polyuronides of alfalfa tops were digested rapidly by rumen microorganisms yielding significant quantities of carbon dioxide, Figure 3. In alfalfa

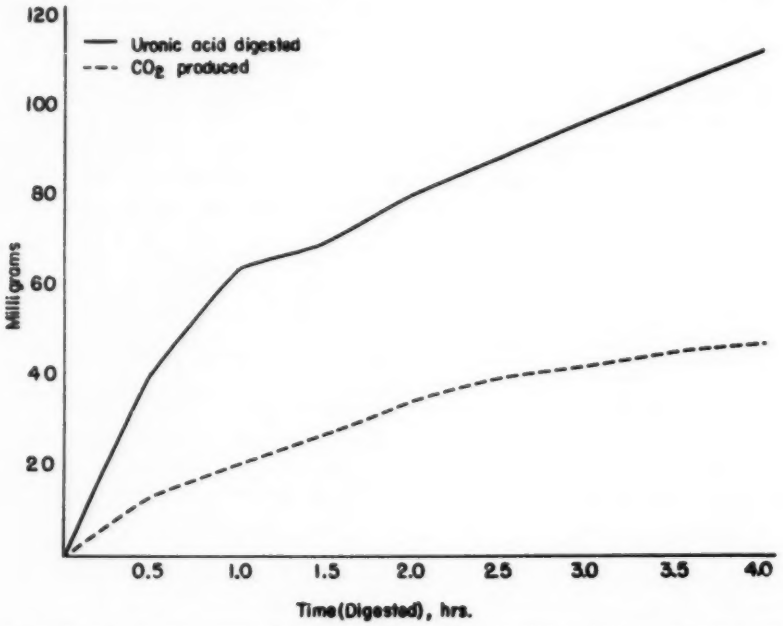

FIG. 3. The disappearance of alfalfa polyuronides digested in vitro with rumen microorganisms and the production of carbon dioxide.

containing 18.2% uronic acid equivalent, 22% was digested within the first 30 min. and 60% within 4 hr. Carbon dioxide production in the in vitro fermentation flasks was highly correlated with the disappearance of the alfalfa polyuronides.

In vivo studies indicated that the polyuronides were readily digested by normal animals. A comparison of the uronic acid content in the ruminal digesta of three cattle is shown, Figure 4, in relation to time of sampling. Steer 75 consistently had small amounts of uronic acid in the digesta, after grazing for 3 hr. After the steer was brought into the barn approximately 20 min. elapsed between the time of last grazing and first sampling of the digesta. It is sug-

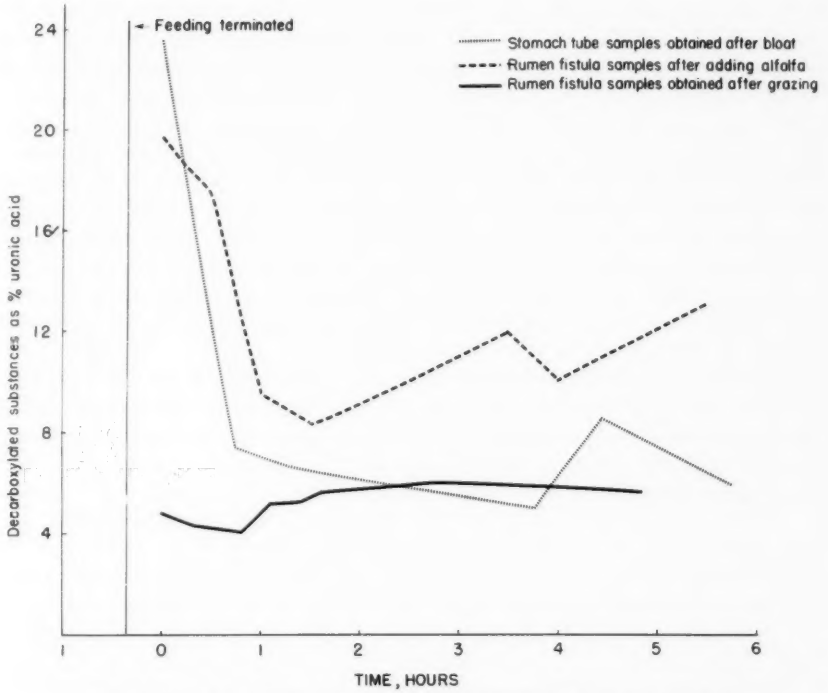

FIG. 4. Hourly changes in the polyuronide content of rumen digesta.

gested, on the basis of these results, that digestion and dilution reduced the polyuronide content of the rumen to between 4 and 6%. On the other hand, digesta obtained from Heifer 15 by means of a stomach tube as bloat was subsiding was high in polyuronide content (23.6%). Within 45 min. it had decreased to 7%, Figure 4. Uronic acid content of the rumen digesta also declined sharply after hand-filling the rumen with 20 lb. of chopped alfalfa mixed with one-fifteenth molar phosphate buffer, but the rate and extent of decline were less marked than in the post-bloat observation on Heifer 15, Figure 4.

DISCUSSION

Expanding froth formation is currently thought to be the primary cause of bloat in cattle grazing legumes (4). Froth arising from ruminal digestion of legumes is a relatively stable mass and consists of actively foaming substances distributed among a dense network of macerated pieces of partly digested plant material (19). In bloated animals the presence of ruminal froth deranges the physiological mechanisms for expulsion of gas (5, 10, 23).

Legumes appear to contribute to the formation of stable froth in three ways: (a) The plant characteristic, which has to do with the manner in which bloat-provoking legumes fracture abruptly across the stem when subjected to chewing

and muscular contractions of the forestomach. These plants break up into short pieces, providing the essential superstructure for stable foam formation in the rumen (19). In contrast, mastication of grasses produces longer pieces of material, with a stringy appearance. (b) The content of plant substances that enhances stable foam formation. It was postulated previously that the pectins in legumes contribute to stable froth formation in the rumens of bloated cattle (7); results presented in Table 1 provide an additional basis for this. Increases in acid-soluble pectic substances were positively related to the periodic occurrence of bloat in cattle grazing Ladino clover, Table 1. Inspection of these data suggests that, with respect to Ladino clover, bloat may be anticipated when the acid-soluble pectin increases to 2% or higher. The grasses contained only 0.26 to 0.68% pectin. Although substantial evidence has been presented correlating pectic substances with bloat incidence, the quantity of pectic substances in stable froth remains to be determined. Nichols (18) recently described in vitro experiments showing that polygalacturonic acid, pectic acid, and sodium pectate increased froth and gas volume. Head (11) reported that the carbohydrate components of ruminal froth were chiefly those found in pectins and hemicelluloses.

No doubt, other plant and animal substances play roles in the foaming of ruminal ingesta. For example, other experimental evidence implicated the cytoplasmic proteins (12), saponins (15), and plant lipids which form bubble coatings (24), as important constituents in ruminal froth formation. However, the plant content of these substances has not been related to the onset of bloating, as was done with pectic substances in Ladino clover. Barrentine (1) found no relationship between the saponin content and the bloat potential of Ladino, alfalfa, and other clovers.

Precursors to ruminal gas formation result in the third plant contribution to the bloat complex. Foam expansion emanating from the rapidly forming gases of microbial decomposition of plant substances may cause pasture bloat to be especially severe, or fatal. Rosen *et al.* (22) stated the general concept of the role of rapid gas formation in alfalfa pasture bloat when they proposed that it resulted from an abundance of substrate available for microbial decarboxylation and the consequent release of carbon dioxide which becomes trapped in the froth caused by saponins and colloids. Fermentation of soluble sugars has been considered a major source of rapid gas formation in the rumen (21), but the plant content of soluble sugars is seldom more than 3%.

From results of this experiment it is concluded that the initial rapid gas production in the rumen of cattle grazing legume pastures comes principally from decarboxylation of polyuronides. They make up 14 to 15% of the total plant matter of legumes but only 6 to 8% of the grasses. The polyuronides are largely galacturonic acid units contained in pectic substances and certain hemicelluloses as part of both leaves and stems. Polyuronides are readily fermented by rumen microorganisms, Figures 3 and 4. Rate of digestion varies with stage of maturity (9). Pressey *et al.* (20) showed that the water-soluble pectin consumed during grazing disappeared readily from the ruminal content,

whereas the remaining pectins were more slowly digested. Also, in in vitro experiments, pectin was metabolized eight times more rapidly than its principal hydrolytic product, galacturonic acid.

Pentosans, relatively high in grasses and low in legumes, Table 4, were digested more slowly than polyuronides.

From these results it is concluded that part of the principal plant materials causing bloat are the acid-soluble pectic substances and readily fermentable polyuronides, in combination with the pulpy plant mass resulting after mastication of legumes.

The grain effects noted are of doubtful practical meaning. The data were interpreted as indicating that the grain feeding altered the pattern of legume intake which diminished slightly but not significantly the number of bloat cases observed.

Where grasses, orchardgrass, and timothy were available only one serious outbreak of bloat occurred. This happened during a three-day pasture period, and an abundance of Ladino clover was also available. This re-emphasizes the usefulness of the grasses in well-managed pasture programs for prevention of bloat. The role of grasses in preventing pasture bloat has been discussed by Cole and Boda (4).

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MILK PRODUCTION AND GROWTH OF IDENTICAL TWIN HEIFERS CALVING FOR THE FIRST TIME AT TWO AND THREE YEARS OF AGE

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SUMMARY

Five identical-twin heifers, calving for the first time at 3 yr., were more persistent in lactation than their mates, which started their second lactation at 3 yr. The early-calving heifers reached a higher peak production than did the late-calving heifers, but ended the lactation with slightly less total milk, on the average. Four- and five-year lactations did not appear to be affected by the age of first calving. Growth or body size was not affected by the different ages of freshening after both groups had completed one or more lactations.

The age at which a dairy heifer first calves is an important factor in herd management efficiency, because at this point the heifer changes from a nonproducing expense item to an income-producing cow. Normally, heifers are bred to calve between 2 and 3 yr. of age. It has been observed that later-calving heifers yield considerably more in their first lactation than do the early-calvers (1, 3, 4, 6, 7, 12, 13). If the increased yield resulting from aging 2 to 3 yr. would pay for the cost of the longer growth period, the age of first calving would be economically unimportant. Another factor to consider is the possible effect of age of first calving on lactations after the first one. This subject has been reviewed recently by Gethin (6).

The most extensive studies of this problem have been reported by Hansson (8), Chapman and Dickerson (3, 4), and Turner (13). Recent reports concerned with age of first calving have also been presented by European workers Bakallo and Lucker (2), Auriol and Grosclaude (1), Höll (9), Østergaard (11), and Zorin and Kochenov (16). These reports have agreed that after the first lactation there was little or no effect of first-calving age upon lactation yields. As a result, early-calving heifers maintained a higher cumulative milk yield at any given age than did late-calving heifers. It was also noted that size at maturity was not affected by age of first calving.

Six pairs of identical- and nine pairs of fraternal-twin heifers were used in Auriol and Grosclaude's research (1). Early-calving twins yielded about the same as their late-calving mates, except for the first lactation. This resulted in 28 to 35% higher total milk yields from the early-calvers by 5 yr. By that age, the pairs were also nearly identical in size.

A theory was advanced by Turner (13), that the production of second-lactation 3-yr.-old heifers may be higher than that of first-lactation 3-yr.-olds, because of previous development and function of the udder in the first lactation of the former. His analyses showed that increase of production due to age within one lactation was about 20 lb. milk fat for each 100-lb. increase in body weight and

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that, on the average, differences in first-lactation yield due to age at calving were nearly all accounted for by body weight differences at this ratio. When production of cows in different lactations was correlated with body weight, the increased yield was about 100 lb. of milk fat for each 100-lb. increase in body weight, the major part being due to age or lactation differences.

The relative influences of age and weight on first-lactation yields have been

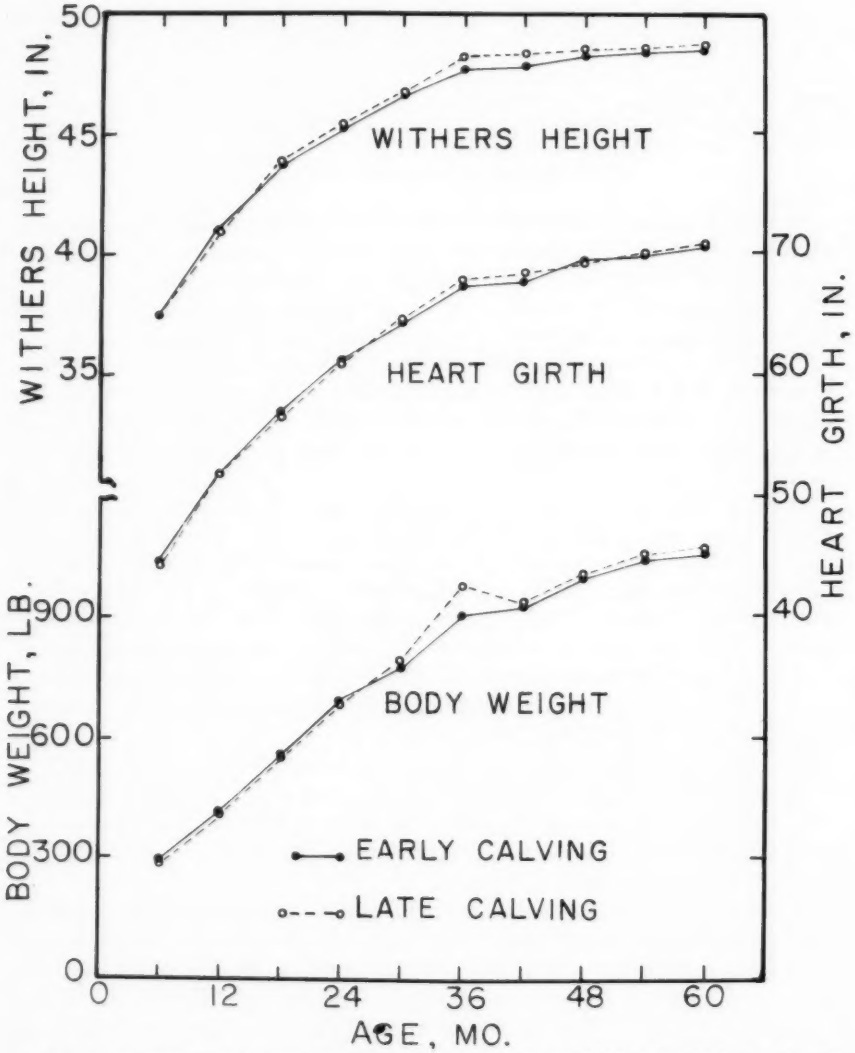

FIG. 1. Average growth curves of five pairs of identical-twin heifers in which one of each pair calved for the first time at 2 yr. and one at 3 yr. of age.

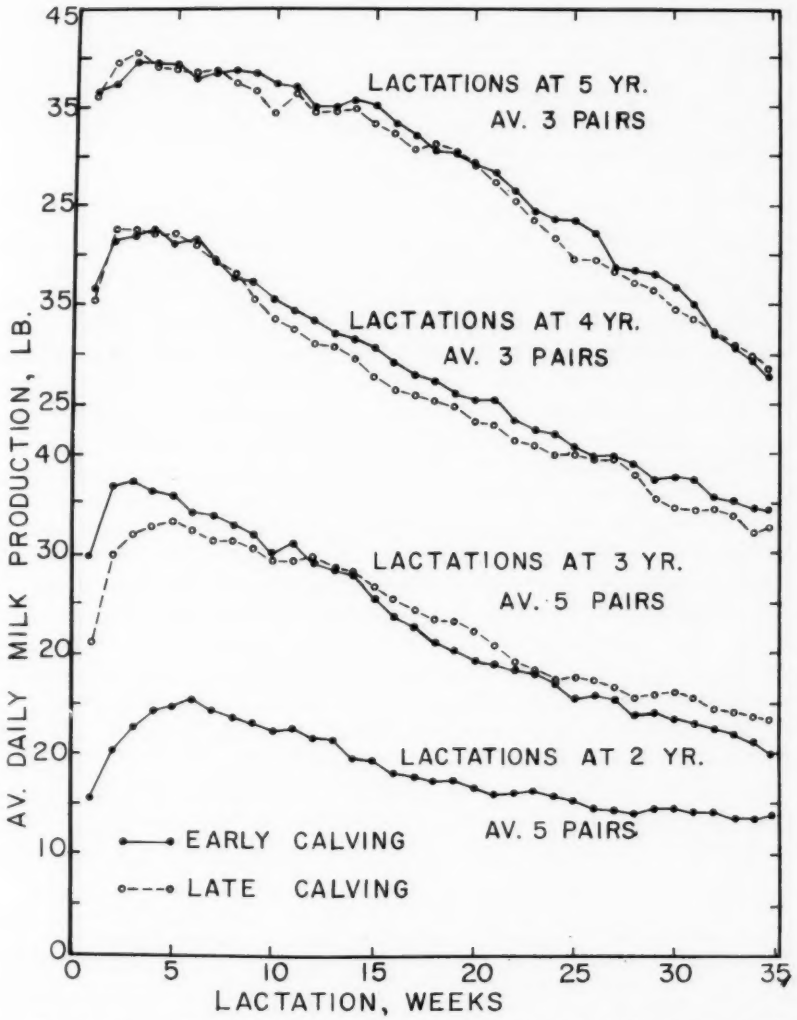

FIG. 2. Average lactation curves of five pairs of identical-twin heifers comparing 2- and 3-yr. ages for first calving.

studied by Miller and McGilliard (10) and Farthing and Legates (5). These studies indicated that effects of age were more important than effects of weight upon actual milk yields. A study of mouse mammary glands by Wada and Turner (15) indicated that the secretory tissue of the mammary gland increased markedly with recurring pregnancies, but was not closely related to increasing body weight.

The precise effects of age at first calving upon lactation can be determined

best by using identical twins. In monozygous twin pairs differences due to genetics are fully controlled. Most environmental differences can be minimized by feeding and handling the twins together and breeding them to calve at nearly the same time (1). This study was made to determine the effect of age of first calving on growth and milk production, using identical twins with initial lactations beginning at 2 and 3 yr. of age.

EXPERIMENTAL PROCEDURE

Five pairs of identical twins used in this experiment were obtained from Tennessee dairy farmers They were all grades, including one Jersey, two Guernsey, one Holstein × Jersey, and one Guernsey × Jersey cross. They were judged to be monozygous on the basis of colors and body conformation. All pairs had identical blood cell antigen types. They were designated for this experiment at about 1 yr. of age. One of each pair was bred to calve at about 2 yr. of age. Heifers in this group (early) calved at 22 to 27 mo. of age, and averaged 24.6 mo. Before this first calving each pair of twins was fed and handled together in a normal manner. They were pastured or fed only hay after about 1 yr. of age. After about 2 yr. of age the milking and nonmilking twins were separated. The milking heifers were fed normal herd rations, including hay, silage, pasture, and adequate concentrate rations. The nonmilking heifers, along with other precalving heifers in the University of Tennessee herd, were fed only roughages. Twins of each pair were bred so they would calve close together at about 3 yr. of age.

Calvings for the 3-yr. lactations were from 34 to 38 mo. of age in the early group and from 33 to 38 mo. in the late group, with each averaging 36.4 mo. at parturition. During this lactation, and subsequently, each pair of twins was stabled together and fed, handled, and milked alike.

Breeding and health difficulties resulted in the loss of two of the pairs from this experiment for later lactation comparisons. The other three pairs subsequently calved together for two more lactations. The average ages at calving

TABLE 1

Ages of individual heifers of the identical twin pairs at parturitions

Twin pair No.	Calving ages and groups						
	2 yr.	3 yr.		4 yr.		5 yr.	
	Early	Early	Late	Early	Late	Early	Late
	(mo.)						
1	26	38	38	50	50	66	66
2	22	35	33	47	47	60	59
3	23	34	36	48	48	59	60
4	27	38	38	(1)	(1)	....	....
5	25	37	37	(2)	(2)	....	....
Av.	24.6	36.4	36.4	48.3	48.3	61.7	61.7

(1) Pair 4 calved more than 6 mo. apart at 4 and 5 yr.; therefore, they were not used for production comparisons.

(2) Pair 5 was eliminated after 3 yr., because one heifer suffered severe coliform mastitis, resulting in abortion.

were 48.3 and 61.7 mo. in each group for the 4- and 5-yr. lactations, respectively. Ages of all experimental calvings are presented in Table 1.

Body weights, height of withers, and heart girth measurements were made each month. The weight of each twice-daily milking was recorded. Composite milk samples were taken one day each week and tested for milk fat and total solids.

RESULTS

Growth. The average body weight, withers height, and heart girth at 6-mo. intervals to 60 mo. are shown in Figure 1 for the five pairs of twins calving initially at 2 or 3 yr. of age. The average growth curves were nearly identical until after 30 mo. At 36 mo. the late-calving group averaged 68 lb. more in body weight, 0.6 in. higher in withers, and 0.8 in. greater in heart girth than their early-calving mates. Because they were not lactating, the late group had grown slightly faster and was in fatter condition than their mates at 3 yr. Nearly all of this difference had disappeared by 42 mo. when both groups were lactating. By 48 to 60 mo. the group averages were again nearly identical.

Lactation. Lactation curves for the first 4 yr. are presented in Figure 2. The 2- and 3-yr. lactation curves give a comparison of first lactations at these respective ages, as well as comparing first and second lactations at 3 yr. The first lactations were more persistent than later lactations. The second-lactation 3-yr.-old heifers started at higher levels of production than their first-lactation mates, and maintained this superiority for 12 wk. However, the slower decline in yield of the first-lactation 3-yr.-old heifers resulted in a greater lactation yield for the late-calving heifers.

The three pairs compared in the 4th and 5th yr. produced nearly the same, on the average. Yields were generally closer together at the beginning of the lactations than at the end. The heifers tended to milk alike within pairs. For this reason, they were generally dried off at the same stage of lactation. The actual lactation periods are presented in Table 2. Pair 4 had poor persistency.

TABLE 2
Lactation periods of individual twin heifers

Twin pair No.	Calving ages and groups						
	2 yr.	3 yr.		4 yr.		5 yr.	
	Early	Early	Late	Early	Late	Early	Late
	(days)						
1	294	294	294	294	294	273	266
2	294	280	294	245	245	231	224
3	294	294	294	294	294	252	238
4	112	140	147	.....	.....	.....	.....
5	294	294	294	.....	.....	.....	.....
Av. 1–3	294	289	294	278	278	252	243
Av. 1–5	258	260	264	.....	.....	.....	.....

All lactations were affected in the 5-yr. lactations by unknown management factors which affected other cows in the herd as well as these experimental twins.

TABLE 3

Individual and group average lactation yields of milk and 4% fat-corrected milk of identical twins comparing early and late first-calving ages

Twin pair No.	Calving ages and groups						
	2 yr.	3 yr.		4 yr.		5 yr.	
	Early	Early	Late	Early	Late	Early	Late
	(lb.)						
Milk							
1	9,569	10,148	12,311	10,478	11,125	8,928	10,557
2	5,036	4,394	4,555	4,242	4,796	5,658	4,889
3	4,765	6,239	5,462	7,511	4,972	6,543	5,126
4	1,530	2,204	2,805	.....	.....	.....	.....
5	4,425	7,408	6,216	.....	.....	.....	.....
Av. 1–3	6,457	6,927	7,443	7,410	6,964	7,043	6,857
Av. 1–5	5,065	6,079	6,270	.....	.....	.....	.....
4% Fat-corrected milk							
1	9,738	10,509	12,769	11,135	11,604	8,650	10,501
2	6,214	5,373	5,707	5,205	5,695	7,076	6,221
3	5,251	6,411	5,650	7,460	5,197	6,545	5,297
4	1,572	2,457	3,327	.....	.....	.....	.....
5	5,175	8,513	7,361	.....	.....	.....	.....
Av. 1–3	7,064	7,431	8,042	7,933	7,499	7,424	7,340
Av. 1–5	5,590	6,653	6,963	.....	.....	.....	.....

The lactation milk yields and fat-corrected milk are presented in Table 3. In some instances, there was less difference within twins in FCM than in actual yield, because the lower-producing twin usually had a higher milk fat test than her mate. The lactation fat yields and fat tests are presented in Table 4.

TABLE 4

Individual and group average lactation yields of milk fat and milk fat tests of identical twins comparing early and late first-calving ages

Twin pair No.	Calving ages and groups						
	2 yr.	3 yr.		4 yr.		5 yr.	
	Early	Early	Late	Early	Late	Early	Late
	(lb.)						
Fat yield							
1	394	430	523	463	477	339	419
2	280	241	259	234	252	321	284
3	223	261	231	297	214	262	216
4	64	105	147	.....	.....	.....	.....
5	227	370	325	.....	.....	.....	.....
Av. 1–3	299	311	338	331	314	307	306
Av. 1–5	238	281	297	.....	.....	.....	.....
	(%)						
Fat tests							
1	4.12	4.24	4.25	4.42	4.29	3.79	3.96
2	5.56	5.48	5.68	5.51	5.25	5.67	5.82
3	4.69	4.18	4.22	3.96	4.30	4.00	4.22
4	4.21	4.79	5.24	.....	.....	.....	.....
5	5.13	4.99	5.23	.....	.....	.....	.....
Av. 1–3	4.79	4.63	4.72	4.63	4.61	4.49	4.67
Av. 1–5	4.74	4.73	4.93	.....	.....	.....	.....

These production data show considerable variation between pairs in response to the different ages of first calving. As an average of five pairs the late-calving 3-yr.-old heifers produced 4.7% more than their mates. Individual pairs varied from 14% less (Pair 5) to 35% more (Pair 4). These wide differences may have been due largely to the disposition of the animals. Pairs 3 and 5 were more nervous and accepted training to the milking routine less readily than the others. For this reason the experience of the first lactation probably gave these early-calvers an advantage over the late-calvers at 3 yr.

DISCUSSION

Results of this experiment agree in most details with those Auriol and Grosclaude (1) obtained with identical twins. They also observed a variation in responses between twin pairs, in that some late-calving heifers produced less at 3 yr. than did their mates in the second lactation at the same age. The group averages of both of these experiments with identical twins have shown slightly higher production at first than at second lactation at 3 yr. of age, even though the second-lactation heifers started the lactation at a higher level. This response does not agree with Turner's (13) theory that lactation experience and mammary gland development are important factors in the increase in production which occurs with age. These results indicate that the level of production may be determined primarily by the age or maturity of the heifer. Since first lactations are more persistent than later ones, the late-calving heifers excel in their first-lactation yield because they maintain higher milk flow during the later months of lactation than do their second-lactation mates. By the next year, when all heifers have one or more lactations, they are producing approximately the same—a level of production determined by their age, inheritance, and previous treatment or condition.

Effects on growth observed in this experiment agreed with previous studies (1, 6, 8). The late-calving heifers were larger at the beginning of their first lactation than were their mates. This size did not express itself as milking potential, since at this time their smaller second-lactation mates were outyielding the late-calving heifers. However, the extra growth may have provided a body reserve which contributed to the increased persistancy they maintained. After both twins of a pair had finished a lactation together, at 48 mo., they were practically the same size. Therefore, no permanent advantage in size can be expected by delaying calving.

Results of this experiment confirm those of previous investigations, which indicate that the main advantage to delayed calving of heifers is to allow for more maturity, resulting in a larger first lactation. Production and growth at comparable ages after this time are not effected by the age of first-calving. This results in the early-calving heifer always having an advantage in total production at any age after her first lactation.

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EFFECT OF MILD HYPERTHYROIDISM UPON SECRETION OF I^{131} IN MILK OF DAIRY CATTLE [1]

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SUMMARY

Six lactating dairy cows were administered 100 μc. I^{131} intrajugularly. Samples of milk were analyzed daily for five days for the content of I^{131} present. During the first 24 hr. from 4.9 to 7.2% of the injected dose of I^{131} appeared in the milk, with a mean of 5.8%. During the five-day period, the total mean percentage of the injected dose increased to 8.2, with a range of 6.9 to 9.2%.

The experiment was repeated with the same cows during a period when each cow was receiving exogenous thyroxine equal to 50% above its individual winter thyroxine secretion rate. In spite of the fact that daily milk secretion increased an average of 22%, the mean percentage of the injected dose of I^{131} appearing in the milk during the first 24 hr. was reduced to 2.9, with a range of 1.9 to 3.9%. The mean total percentage of I^{131} appearing in the milk during five days was 4.8, with a range of 3.4 to 6.0%. The per cent decline in I^{131} in the milk followed an exponential course in both experiments.

Mild hyperthyroidism thus reduced the I^{131} secretion in milk 50% the first 24 hr., and 41% during the five-day period. It was pointed out that dairy cattle transfer relatively low amounts of I^{131} to their milk and that this amount is further reduced by mild hyperthyroidism.

It has been shown in a number of species that I^{131} is trapped by the mammary gland and secreted into milk (2, 5). However, there appear to be great species differences in the amount of I^{131} secreted into milk. In rats (2) and mice (6) the percentage of an injected dose of I^{131} transferred to the milk is high (mouse—86%, rat—37.8%). Similarly in the dairy goat, the trapping of I^{131} by the mammary gland has been reported to vary from 25 to 50% (1, 5, 7). In these species, it has been suggested that the thyroid gland might not compete successfully with the mammary gland when the iodine content of the feed was limited, resulting in a reduced thyroxine secretion rate (2, 5).

In the dairy cow (3), it was reported that about 6% of the I^{131} (injected or orally administered) was secreted into the milk during seven days. This observation is of great interest from the standpoint of health physics in relation to I^{131} fall-out and in regard to the time the milk from cows receiving I^{131} should be discarded. The low transfer of I^{131} to the milk indicates the relative safety of cow's milk as a food if I^{131} fall-out should occur.

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This experiment was designed to determine the percentage of I^{131} present in the milk of cows whose thyroxine secretion rate was being determined, in order to discard milk containing measurable amounts of I^{131}. In addition, it was possible to study the influence of mild hyperthyroidism (50% above their winter thyroxine secretion rate) upon I^{131} in the milk secreted.

EXPERIMENTAL PROCEDURE

Six lactating cows of several breeds, kept in a dry lot with access to an iodized salt block, were used. One hundred microcuries of I^{131} was injected intrajugularly into each animal and the first sample of milk was collected 3 hr. later. Samples of morning and evening milk were mixed and a representative sample was then taken at 24-hr. intervals for five days. Milk samples were analyzed for I^{131} activity at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. One hundred milliliters of milk was diluted with 3.5 liters of distilled water. An appropriate aliquot sample was then counted for I^{131}, using a 4-in. by 4-in. NaI (thallium-activated) crystal. Efficiency of I^{131} counting was 6.3%.

TABLE 1

I^{131} secretion in milk of cows during control and thyroxine treatment periods (Percentage I^{131} dose in milk)

Breed and No.	Control period			Thyroxine period		
	Daily milk yield per week	Percentage I^{131} in milk		Daily milk yield per week	Percentage I^{131} in milk	
		24 hr.	Five days		24 hr.	Five days
Holstein	*(lb.)*			*(lb.)*		
869	17.5	5.7	8.5	21.8	2.8	4.9
870	21.6	4.9	6.9	26.6	3.2	5.1
873	21.5	5.3	9.2	25.4	3.9	6.0
Brown Swiss						
24	20.6	7.2	9.2	26.8	1.9	3.4
25	26.6	5.8	7.3	32.0	2.3	4.7
Jersey						
630	18.5	6.0	8.1	21.0	3.1	5.0
Mean	21.0	5.8	8.2	25.6	2.9	4.8

During the experimental period in late spring and early summer, I^{131} was injected into the same cows between 3 and 6 wk. after the cows were subjected to thyroxine treatment at a level 50% above their estimated thyroxine secretion rate.

RESULTS

During the control period, from 4.9 to 7.2% of the injected dose of I^{131} appeared in the milk during the first 24-hr. period (Table 1). The mean of the six cows was 5.8%. During a five-day period, the total mean percentage of the injected dose increased to 8.2, with a range of 6.9 to 9.2%. Thus, the increase

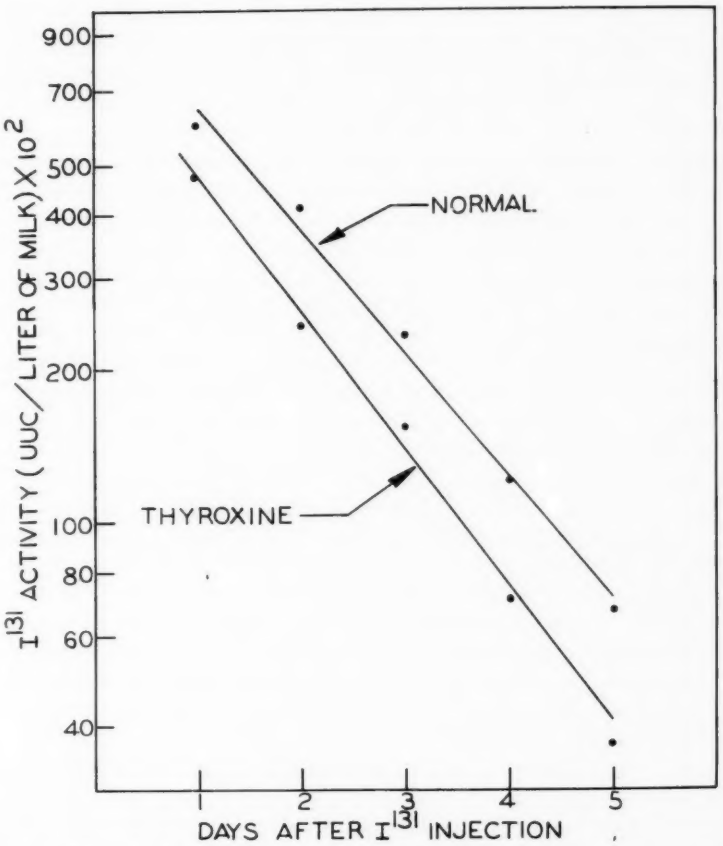

FIG. 1. The mean percentage of I^{131} appearing in the milk of six dairy cows each day for five days after intravenous injection of I^{131}. In same cows receiving thyroxine daily at level 50% above their winter secretion rate, the amount of I^{131} appearing in milk was reduced.

in I^{131} taken up by the mammary glands during the last four days of the test period was only 2.4%.

During the experimental period, the cows each received exogenous thyroxine in an amount equal to 50% above their individual winter thyroxine secretion rates. The mean milk production during the control period was 21.0 lb. This increased during the experimental period to a mean of 25.6 lb., or 22%.

During the first 24 hr. of the experimental period, the mean percentage of the injected dose of I^{131} present in the milk was 2.9, with a range of 1.9 to 3.9%. The mean total percentage of the I^{131} appearing in the milk during five days was 4.8, with a range of 3.4 to 6.0%. The per cent decline in I^{131} in the milk followed an exponential course in both experiments.

DISCUSSION

The present data confirm previous observations with dairy cattle that relatively small amounts of I^{131} are trapped by the mammary glands and secreted into milk. These observations differ from those of Lengemann and Swanson (3), in that only a single injection of I^{131} was made, in contrast to daily oral administration in the previous experiment. However, the amount of I^{131} secreted into milk in the first 24 hr. in the present experiment was quite similar to the amount secreted per 24 hr. after continuous administration.

These observations in dairy cattle are in marked contrast to observations with goats and rodents, which have shown that the mammary glands of these animals trap and secrete much larger amounts of I^{131} into the milk. It might be suggested that the species differences observed are due to the greater secretory activity of the mammary glands of these animals in relation to body weight. Reineke (5) pointed out that in goats the I^{131} concentration in the milk was more than ten times that in blood serum, indicating an active process in mammary tissue for the secretion of iodide.

In the study of the thyroxine secretion rate of lactating dairy cattle, a single injection of I^{131} is administered (4). The maximal permissible amount of I^{131} in the milk of dairy cattle recommended by the National Council for Radiation Protection is 2,000 micromicrocuries per liter of milk. On the fifth day, the amount of I^{131} present in the milk of normal cows had been reduced to three times the maximum permissible amount; whereas, under mild hyperthyroidism the reduction was to about 1.5 times. Thus, on Days 6 to 8, the amount of I^{131} present in milk would slightly exceed the maximum permissible amount. If it is mixed with the milk of a large herd, the I^{131} in the total milk would be far below the permissible amount.

In discussing the effect of mild hyperthyroidism upon the uptake and secretion of I^{131} into the milk, it should be pointed out first that the thyroxine administered would prevent the uptake of I^{131} by the thyroid gland, so that this pathway of removal of I^{131} from the blood would be blocked. The other major pathways of I^{131} would be urine and feces, with the major portion being eliminated in the urine (3). The second effect of thyroxine was to stimulate increased milk secretion.

In spite of these effects of thyroxine, it was observed that the percentage of the injected dose of I^{131} during the first 24 hr. was reduced from 5.8 to 2.9, or 50%, and from 8.2 to 4.8, or 41%, for the five-day period. It is thus evident that mild hyperthyroidism tends to reduce the uptake of I^{131} by the mammary gland cells and to reduce the secretion into the milk. It is logical to conclude that normal cows in the range of higher thyroxine secretion rates would tend also to secrete less I^{131} into their milk.

Since less I^{131} is secreted into the milk during mild hyperthyroidism, and since I^{131} is not collected by the thyroid gland, it is suggested that mild hyperthyroidism tends to increase the rate of removal of I^{131} by the kidney and the liver (bile).

In the event that nuclear fall-out should increase in the future, it is reassuring to note that the milk of the dairy cow would contain relatively little I^{131} and that milk from cows of higher thyroxine secretion rates would tend to have lower I^{131} content.

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LUTEAL FUNCTION AND EMBRYO SURVIVAL IN THE BOVINE[1]

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SUMMARY

The ability of oxytocin injections to inhibit the development of the corpus luteum in the bovine was utilized to study the relationship between progesterone content of luteal tissue and embryo survival at 15 days after insemination in 70 Holstein heifers.

Twenty of 27 untreated heifers (74.1%) had normal embryos and the average total progesterone in their corpora lutea was 270.2 μg. The corpora lutea of the seven untreated heifers from which embryos were not recovered had an average of 304.1 μg. of total progesterone. Eighteen of the 43 heifers (41.9%) injected subcutaneously with 7 U.S.P. units of oxytocin per hundred pounds of body weight per day had normal embryos and the average total progesterone content of their corpora lutea was 310.6 μg. The total progesterone levels in the corpora lutea were not significantly different among these three groups of heifers.

Fifteen oxytocin-treated heifers had a precocious ovulation prior to the 15th day and no embryos were recovered from this group; their corpora lutea contained only an average of 45.8 μg. of total progesterone ($P < 0.01$). The remaining ten oxytocin-treated heifers had no embryos and their corpora lutea also contained significantly ($P < 0.05$) less total progesterone (154.1 μg.) than the treated heifers having embryos.

The level of Δ^4-pregnene-20-β-ol-3-one was significantly ($P < 0.01$) higher in the corpora lutea of the untreated animals without embryos than in any other group.

The data suggest that a threshold level of progesterone production represented by a luteal progesterone level of the order of 100 μg. is necessary for embryo survival at the 15th day.

The four of 15 oxytocin-treated heifers that ovulated precociously without showing signs of estrus had little or no measurable progesterone in their corpora lutea.

Early embryo mortality has been a major cause of infertility in every species in which the problem has been studied (13). Its importance in the bovine, especially in the repeat breeder cow, has been demonstrated (3, 11, 12).

The importance of this problem makes a study involving the quantitative relationships between progesterone levels and embryo survival of particular interest. The discovery by Armstrong and Hansel (2), that oxytocin was capable of inhibiting the development and function of the corpus luteum in dairy heifers, even to the point of causing precocious estrus and ovulation, provided an experimental tool by which the corpus luteum might be partially or completely inhibited in otherwise normal animals. This tool has been used in the present studies to investigate the relationships between progesterone level in the corpus luteum and embryo survival in dairy heifers.

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EXPERIMENTAL PROCEDURE

Each of the 70 Holstein heifers used in the experiment was bred artificially at an estrus following a cycle of normal length (17-24 days) with 2 ml. of semen containing 20 million motile sperm. The semen was obtained from bulls in routine use at the New York Artificial Breeders' Cooperative, and was extended in a medium containing 50% egg yolk and 50% buffer consisting of 2.9% citrate and 0.6% sulfanilamide, with 500 units of penicillin and 500 μg. of dihydrostreptomycin added to each milliliter of final extender.

Twenty-seven of the bred heifers received no further treatment and were slaughtered on the morning of the 15th day following estrus. The remaining 43 heifers were each injected subcutaneously with 7 U.S.P. units of oxytocin [3] per hundred pounds of body weight daily for 14 days, commencing on the day following the initiation of estrus, and were slaughtered on the morning of the 15th day.

At the time of slaughter the corpora lutea were removed from the ovaries, weighed, and split into approximately equal parts. One half of each corpus luteum was fixed in Bouin's solution for future histological study; the remaining half was frozen in dry ice and stored at $-15°$ C. in 95% ethanol in a sealed container. The ovaries, adrenals, and thyroids were weighed and fixed in Bouin's solution. The pituitaries were dissected free, split mid-sagittally, and fixed in modified Bouin's solution. The oviducts were removed from the reproductive tracts and placed in Bouin's solution.

The uteri were placed in an insulated box maintained at approximately 37° C. and transported to the laboratory. The embryos were flushed from the cervical end of the uteri by introducing Locke's solution in 10-ml. aliquots into the tip of each uterine horn. These embryos were quickly measured, photographed, and placed in Helly's fixative. A cross-sectional segment of the uterine horn corresponding to the ovary containing the active corpus luteum was then removed and fixed in Bouin's solution. This segment was removed from a point midway between the cervix and the tubo-uterine junction.

After fixation all tissues were stored in 70% ethanol prior to sectioning. The progesterone determinations involved six major steps as follows:

Step 1. Homogenization of the luteal tissue containing 0.01 μc. of added C^{14}-4-progesterone and extraction of the progesterone by refluxing twice with 50-ml. aliquots of 95% ethanol.

Step 2. Alumina adsorption column chromatography of the filtered and dried ethanol extract.

Step 3. Eight-transfer countercurrent distribution of the progesterone-containing eluant in a n-pentane–70% methanol system.

Step 4. Descending paper chromatography of the progesterone containing fraction at room temperature in a hexane–80% methanol system.

Step 5. Identification of the progestational compounds by photography under ultraviolet light, and their elution from the paper.

[3] Armour's Purified Oxytocic Principle.

Step 6. Spectrophotometric measurement of the sample and measurement of the activity of the C^{14}-4-progesterone recovered in a liquid scintillation spectrometer, followed by calculation of the progesterone in the sample of luteal tissue.

Since the method used appears to give slightly higher progesterone values than others have reported, and some of the procedures used have not previously been published, pertinent details concerning each step are included. All solvents were redistilled prior to their use, and twice-distilled water used for all dilutions.

Step 1. Luteal tissue, usually weighing about 0.5 g., and a proportionate portion of the ethanol in which it was stored, were transferred to a tissue grinder. In approximately half of the determinations 0.01 μc. of C^{14}-4-progesterone was added at this point. The sample was then homogenized and subsequently refluxed with two 50-ml. portions of 95% ethanol for 2 and 1 hr., respectively. The supernatant fluid was filtered through Whatman No. 42 filter paper, and dried at 70° C. under reduced pressure, on a rotary evaporator equipped with a trap to prevent contamination of the sample with lubricants from the evaporator.

Step 2. The dried samples were subjected to alumina adsorption column chromatography as outlined by Duncan (8), with the exception that an additional 40 ml. of chloroform eluant was collected following the 25% chlorohexane eluant.

Step 3. The samples were dried as previously described and subjected to an eight-transfer countercurrent distribution system (5, 6) consisting of n-pentane and 70% methanol (8). To retain the progesterone metabolite Δ^4-pregnene-20-β-ol-3-one (14, 17), which has progestational activity in the human (10, 18), the methanol fractions of Tubes 1 and 2 and the entire contents of Tubes 3-8 inclusive were collected.

Step 4. The samples were dried as before and transferred to 10- by 76-mm. tubes with two aliquots of 0.5 ml. and one of 1.0 ml. of absolute methanol. The contents of the tubes were dried under a stream of nitrogen and the samples were concentrated on the bottoms of the tubes by adding and drying 0.3 ml., followed by 0.1 ml. of absolute methanol. The samples were then spotted on a strip of Whatman No. 1 filter paper for descending chromatography, as described by Bush (4). Hexane was used for the mobile phase rather than ligroine, as described by Bush (4), and the chromatography was carried out at 23° C. rather than at 37° C. Each sample was chromatographed along with a blank paper strip upon which 100 lambda of absolute methanol was spotted and a similar paper strip upon which pure progesterone of a standard concentration was spotted.

Step 5. After chromatography, the paper strips were photographed under ultraviolet light, and the progesterone and Δ^4-pregnene-20-β-ol-3-one spots located by matching the paper strips to the photograph. The spots were then cut out and the hormones eluted in a special drip-type eluter similar to that used by Zander and Simmer (19).

Step 6. The eluants were dried as previously described and transferred in absolute ethanol to semimicro cuvettes and measured in a Model DU Beckman spectrophotometer. Optical density readings were made for each sample at 220, 240, and 260 mμ. Each corpus luteum sample was read against a blank prepared by eluting an area of paper from the blank chromatogram similar in size and Rf value to that used for the unknown. The progesterone content of the sample was calculated by means of a standard curve, after applying Allen's (1) correction to the optical density reading.

The percentage recovery of the sample, as well as the percentage of progesterone lost at each major step, was determined by counting the activity of the C^{14}-4-progesterone (Nuclear-Chicago, CFA × 148, Sp. Act. 5 mc/m mole) in a Tricarb Liquid Scintillation Spectrometer (Packard Instrument Co., La Grange, Ill.) in D.P.O.[4] solution. All values reported are corrected for losses during the procedure.[5]

RESULTS

Embryos recovered were considered normal if no abnormality could be detected either grossly or after microscopic study. Results of the embryo recoveries are summarized in Table 1; this shows that the percentage of embryos recovered from the oxytocin-treated heifers was significantly reduced.

TABLE 1

Embryos recovvered at 15 days after insemination from oxytocin-treated and untreated heifers

Treatment	No. inseminated	No. with normal embryos	Per cent with normal embryos
Untreated	27	20	74.1
Oxytocin-treated	43	18	41.9[a]
Did not ovulate before Day 15	28	18	64.3
Ovulated before Day 15	15	0	
Total	70	38	

[a] Chi-square = 5.70; P = 0.01-0.02, when analyzed by a 2 × 2 contingency table.

Perhaps the most remarkable result shown is that 15 of the 43 oxytocin-treated heifers ovulated precociously despite the low doses of oxytocin administered. None of these 15 heifers had embryos; 11 of them came in estrus before the 15th day and four did not do so.

When the 28 oxytocin-treated heifers that did not have a precocious ovulation are considered alone, the percentage of embryos recovered (64.3) was not significantly different from the percentage obtained for the untreated heifers. Only three degenerate embryos were recovered; in all other cases the embryos were either normal or absent.

The average total progesterone in the corpora lutea of the treated and un-

[4] Consisted of 4 g. of 2,5-diphenyloxazole (P.P.O.) and 100 mg. of 1,4,bis-2-5-p-hexyloxazolyl-benzene (P.O.P.O.P.) made up to one liter with toluene.

[5] Further details concerning the methods and equipment used are available on request.

treated animals with and without embryos, and in the corpora lutea of the treated animals that had a precocious ovulation, is shown in Table 2. The

TABLE 2

Average total progesterone[a] content of the corpora lutea of untreated and oxytocin-treated heifers 15 days after insemination

Group	Treated—without embryos—precocious ovulations	Treated—without embryos	Untreated—with embryos	Untreated—without embryos	Treated—with embryos
No. of animals	14	10	19	6	16
Average (μg.)	45.8[b]	154.1	270.2	304.1	310.6
Duncan's M.R.T.[c] 1%					
5%					

[a] The amounts of Δ^4-pregnene-20-β-ol-3-one and progesterone have been combined for each heifer in each group.

[b] Includes the total progesterone in the initial corpus luteum and the one formed after the precocious ovulation.

[c] Treatments not underscored by a continuous line are significantly different (7).

average total progesterone in the corpora lutea of all treated animals was 178.8 μg., as compared to 278.3 μg. for all untreated animals, a statistically significant difference ($P = 0.01$-0.025).

However, both the treated heifers with embryos and the untreated heifers without embryos had as much total progesterone in their corpora lutea as the untreated heifers with embryos. The progesterone content of the corpora lutea of both the treated groups without embryos was significantly lower than that of the treated animals having embryos. Extremely low values were obtained for most heifers that had a precocious ovulation.

The average levels of Δ^4-pregnene-20-β-ol-3-one for the treated and untreated groups are shown in Table 3. The salient feature of these data is the significantly

TABLE 3

Average Δ^4-pregnene-20-β-ol-3-one content of the corpora lutea of untreated and oxytocin-treated heifers 15 days after insemination

Group	Treated—without embryos—precocious ovulations	Treated—without embryos	Untreated—with embryos	Treated—with embryos	Untreated—without embryos
No. of animals	14	10	18	14	6
Average (μg.)	1.4[a]	26.6	58.5	63.1	105.8
Duncan's M.R.T.[b] 1%					
5%					

[a] Includes the value for the initial corpus luteum and for the one formed after the precocious ovulation.

[b] Treatments not underscored by a continuous line are significantly different (7).

elevated levels of this metabolite found in the corpora lutea of the untreated animals without embryos. Both treated groups without embryos had low levels of this metabolite in their corpora lutea, and the levels in the corpora lutea of the treated and untreated heifers with embryos were about the same.

Table 4 shows the total progesterone levels found per gram of luteal tissue.

TABLE 4

Average total progesterone per gram of luteal tissue in untreated and oxytocin-treated heifers 15 days after insemination

Group	Treated—without embryos—precocious ovulations	Treated—without embryos	Untreated—with embryos	Treated—with embryos	Untreated—without embryos
No. of animals	14	10	19	16	6
Average (μg.)	15.3[a]	32.8	56.9	61.2	68.4
Duncan's M.R.T.[b] 1%					
5%					

[a] Includes the total progesterone in the initial corpus luteum and the one formed after the precocious ovulation.

[b] Treatments not underscored by a continuous line are significantly different (7).

Again, the levels were lowered in the tissues from the treated heifers without embryos and those having precocious ovulations, but did not differ among the untreated heifers with and without embryos and the treated heifers with embryos.

DISCUSSION

The techniques used for the progesterone determinations were satisfactory. Recoveries of the added C^{14}-4-progesterone varied only from 70-80%, which compares favorably with the variation reported by Loy *et al.* (15). The paper chromatography system used gave distinct spots for both progestational compounds and there was little evidence of the trailings noted by Edgar (9) as a result of room-temperature chromatography (Figure 1).

Major losses in the determination occurred during adsorption column chromatography (approximately 5%), during the countercurrent distribution (approximately 5%), and during the paper chromatography (approximately 10%). None of the activity from the C^{14}-4-progesterone appeared in the Δ^4-pregnene-20-β-ol-3-one elutions, indicating that progesterone was not converted into this metabolite during the procedure.

Levels of total progesterone found were higher than those reported by Mares and Casida (16) for normally cycling heifers at the 15th day. This difference appears to be mainly due to the higher levels of Δ^4-pregnene-20-β-ol-3-one found in the present study.

Data obtained indicate that embryo development can proceed normally when the level of progesterone in the corpus luteum is above some threshold value, and that it fails to occur at subthreshold values. No embryos were recovered

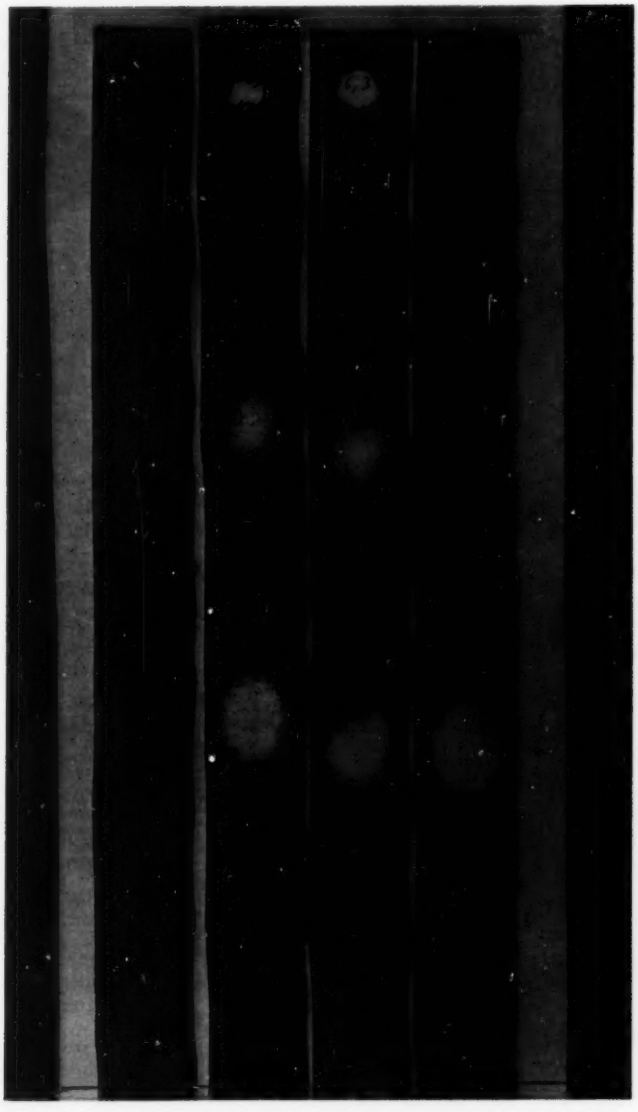

FIG. 1. Ultraviolet light photographs of the chromatograms of luteal extracts from two heifers (center), pure progesterone (right), and a blank strip (left). The upper spots represent the points at which the extracts were applied, the middle ones are Δ^4-pregnene-20-β-ol-3-one, and the lower ones are progesterone. The solvent fronts are just above the matching notches cut into the strips at the bottom.

from heifers having less than 100 μg. of total progesterone in their corpora lutea, except in one doubtful case, in which there was reason to believe that the determination was erroneous as a result of contamination of the sample in the laboratory. Two of the three degenerate embryos found were from heifers whose corpora lutea contained less than 100 μg. of total progesterone. A normal level of progesterone was found in the corpus luteum of the third heifer with a degenerate embryo. Sixteen of the 24 treated heifers without embryos (Table 2) had luteal progesterone levels below 100 μg.; four had levels slightly in excess of 100 μg., and the remaining four had normal levels of 222-341 μg. These observations all suggest that a threshold level of progesterone production represented by a luteal progesterone level of the order of 100 μg. is necessary for embryo survival at the 15th day.

The response of the corpus luteum to the oxytocin injections seems to be an all-or-none phenomenon. If the corpus luteum is successfully inhibited by the injections, extremely low levels of progesterone result, embryos do not survive, and the animal is likely to ovulate precociously. On the other hand, if the oxytocin injections fail to inhibit the corpus luteum, normal amounts of progesterone appear to be produced and the embryo has a normal or nearly normal chance of survival.

The four oxytocin-treated heifers having precocious ovulations without coming in estrus are of particular interest, since three of these had no measurable progesterone in their corpora lutea and the fourth had only a total of 25.1 μg. A low level of progesterone may play a role in the normal manifestation of estrus, as previously suggested.

The elevated levels of Δ^4-pregnene-20-β-ol-3-one in the corpora lutea of the heifers in the control group not producing embryos can not be explained. This may simply represent some change in the metabolism of the luteal tissue, leading to regression of the corpus luteum before the animal's return to estrus, or it may represent some fundamental error of metabolism associated with embryonic death.

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LUTEAL FUNCTION AS RELATED TO PITUITARY AND OVARIAN CYTOLOGY AND EMBRYO DEVELOPMENT IN THE BOVINE [1]

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SUMMARY

Embryos recovered 15 days after insemination from 20 of 27 untreated Holstein heifers and those recovered from 18 of 43 heifers treated daily with 7 U.S.P. units of oxytocin per hundredweight per day did not differ in total length, embryonic disc size, or mesodermal development attained. No embryos were recovered from animals with less than 100 $\mu g.$ of total progesterone in their corpora lutea; normal embryo development appeared possible when the level of luteal progesterone was above this figure. The weights of the corpora lutea of the 15 oxytocin-treated heifers which had precocious ovulations were significantly ($P < 0.01$) reduced, and these corpora contained few normal luteal cells. Ten cystic corpora lutea were found in treated heifers and one in an untreated heifer. These cystic corpora lutea were all formed after ovulation; the cysts were 7 mm. or more in diameter and were separated from the surrounding luteal cells by fibrous tissue. Only two heifers with cystic corpora lutea had embryos. Low levels of progesterone were found in cystic corpora lutea, except in one heifer that had a normal embryo. Although an occasional cystic corpus luteum can produce enough progesterone to maintain an embryo until the 15th day, most of them appear to produce little progesterone and they are not likely to be associated with live embryos. The gonadotrophin-producing delta cells in the anterior pituitary were degranulated in some of the heifers which ovulated precociously and in the control heifers that did not have embryos.

In a previous paper, Staples and Hansel (10) studied the effects of altering the level of progesterone in the corpus luteum by oxytocin injections on embryo survival at 15 days in 70 Holstein heifers. In the present paper the effect of varying luteal progesterone levels on the rate of embryo development and on pituitary, ovarian, and uterine cytology in these same heifers is considered.

The heifers in this experiment were slaughtered at the 15th day after breeding, because it is at this time that the trophoblast normally elongates at a very rapid rate, mesoderm formation begins, and the embryonic disc becomes oval in shape. It was hoped that in cases where embryonic development was inhibited, spherical blastocysts having no mesoderm and round discs would be found. The relative simplicity of the embryo at this stage, as well as the fact that luteal progesterone levels would still be maximal in those heifers which failed to conceive, were additional factors which led to choice of the 15th day.

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EXPERIMENTAL PROCEDURE

The details of the experiment concerning the oxytocin injections, the methods used to recover the embryos, the methods used in the progesterone determinations, and the collection and fixation of tissues have been described (10).

The embryos were removed from Helly's fluid after fixation for 8-10 hr., dehydrated for 15 min. each in 35 and 50% ethanol, and stored in 70% ethanol. The segment of the embryo containing the embryonic disc was then cut from the remainder, blocked in paraffin, cut serially in cross section at 10 μ, and stained with Harris' hemotoxylin and eosin.

The uterine cross-sectional segments were blocked in paraffin, sectioned at 6 μ, and stained with Harris' hematoxylin and eosin. Surface epithelial cell heights for 50 cells evenly distributed about the lumen were measured with a micrometer and averaged for each animal. The uterine cross section for each heifer was photographed and the total area of the endometrium determined with the aid of a planimeter. Additionally, photographs of the endometrium approximately midway between the lumen and the myometrium were made for each heifer, and the area of every gland on each photograph was determined by planimetry. In this way, calculations of endometrial cross-sectional areas, average gland sizes, and the numbers of glands in the cross-sectional areas were made.

The ovaries, oviducts, thyroids, and the luteal tissue not used for progesterone determinations were sectioned and stained in the same manner. The pituitary glands were sectioned at 6 μ and stained by the periodic acid-Schiff technique of Pearse (9) as modified by Jubb (8), and the aldehyde-fuchsin method of Gomori (5) as modified by Halmi (7). Thyroid epithelial cell heights were measured by methods described previously (2).

RESULTS

The embryos. The average embryo ages and measurements are shown in Table 1. The age of each embryo was calculated as being equal to the hours elapsing between the time the heifer was first observed in standing estrus and the time of slaughter minus 29.5 hr. Figure 1 shows a histological section of the embryonic disc of a normal 15-day embryo.

TABLE 1

Embryo ages and measurements at 15 days

Measurement	From untreated heifers		From oxytocin-treated heifers		Probability of significance of difference
	No.	Mean	No.	Mean	
Embryo age *(hr.)*	20	333.5	18	333.2	
Total length *(mm.)*	19	44.2	18	76.2	0.15–0.20
Disc length *(mm.)*	12	0.412	11	0.470	0.25–0.30
Incidence of mesoderm development	18	5	18	6	0.98–0.99

FIG. 1. Histological section of the embryonic disc of a normal heifer, showing the initiation of mesoderm formation to the right. Cut at 10 μ ($\times$ 345).

Neither the embryo lengths nor the lengths of the embryonic discs were significantly different in the treated and untreated groups. As expected, the range of the lengths of the trophoblasts was very large in both groups. In the untreated animals the trophoblast lengths ranged from 6 to 180 mm., as compared to a range of 6 to 220 mm. for the treated heifers. Neither embryo lengths nor embryonic disc lengths were significantly correlated with total progesterone levels in the corpora lutea.

Mesoderm formation had begun in about an equal number of cases in embryos from treated and untreated heifers. Embryos having mesoderm were longer ($P < 0.005$) and had larger discs ($P < 0.005$) than those without mesoderm. The corpora lutea in the ovaries of heifers having embryos with mesoderm were larger and contained more total progesterone, although these differences were not statistically significant ($P = 0.05$-0.1).

Three abnormal embryos were recovered, two from oxytocin-treated heifers and one from an untreated heifer. The embryonic discs had begun to disintegrate in the two embryos from oxytocin-treated heifers. Considerable disinte-

gration of the trophoblast had also occurred in the embryo from the untreated heifer, and the embryonic disc was not seen.

The binucleated giant cells of the trophoblast described by Wimsatt (11) in 17-day embryos were present in embryos from each group. These cells, which produce the lining of the maternal crypts (12), become binucleate following mitosis without cytoplasmic cleavage. Greenstein *et al.* (6) suggested that they may play a role in stimulating the maintentance of the corpus luteum of pregnancy in the bovine.

The corpora lutea. The average weight of luteal tissue (2.66 g.) of the treated heifers that ovulated prior to the 15th day was significantly lower ($P < 0.01$) than in any other group. This difference is all the more remarkable because the weights of two corpora lutea (the initial one and the one formed after the precocious ovulation) were included in each value. Average corpus luteum weights in the remaining four groups (treated, with and without embryos, and untreated with and without embryos) ranged from 4.43 to 5.01 g. and were not significantly different from one another. Corpus luteum weights and total progesterone levels in the luteal tissue were not significantly correlated ($r = 0.145$).

Histological studies of the corpora lutea revealed differences which, in some cases, could be related to differences in progesterone content of the luteal tissue. The corpora lutea of the treated heifers which had precocious ovulations contained very little progesterone and consisted mainly of connective tissue elements and few normal luteal cells. The larger of the two corpora lutea present in each of these animals was the one formed after the ovulation at which the heifer was inseminated, and it invariably contained a few more normal luteal cells than the smaller corpus luteum formed after the second ovulation.

No significant differences were found among the progesterone contents of the corpora lutea from the untreated heifers with embryos, the untreated heifers without embryos, and the treated heifers with embryos, nor were any differences in these corpora lutea noted on histological examination. All contained large numbers of the Type I and Type II cells described by Foley and Greenstein (4). Although the corpora lutea of the treated heifers which did not ovulate precociously and which had no embryos contained less progesterone on the average than the corpora lutea of heifers with embryos, no clear-cut histological differences were observed, except in a few of the heifers which had very low levels of progesterone. It was also impossible to distinguish any histological features that might be related to the elevated levels of Δ^1-pregnene-20-β-ol-3-one in the corpora lutea of the untreated heifers without embryos.

A total of 11 cystic corpora lutea was found in the 70 heifers, and ten of the 11 were in the ovaries of the treated heifers. The diameters of the antra in these corpora varied from 7 to 20 mm., and a fibrous lining was present in all cases. All but one contained fluid. Only two of the 11 heifers with cystic corpora lutea had embryos. These two corpora lutea are of particular interest since, unlike the others, they contained a fairly high proportion of normal luteal cells and one of the two was also found to contain a normal level of progesterone.

Unfortunately, the sample for the progesterone determination for the second was lost in a laboratory accident.

Very low levels of progesterone were found in the remaining nine cystic corpora lutea, and on histological examination they were found to contain few normal luteal cells. It is also worth noting that of the 15 heifers which ovulated precociously, four failed to come into estrus. Three of these four heifers had cystic corpora lutea. Three of the four also had no measurable progesterone in their corpora lutea, and the fourth had a total of only 25.1 μg. of progesterone.

The uteri and oviducts. Study of the uterine tissues revealed significant differences between groups in the size of the uterine glands (Table 2), in the cross sectional areas of the endometria (Table 3) and in the endometrial surface epithelial cell heights (Table 4).

TABLE 2

Average endometrial gland sizes in normal and oxytocin-treated heifers 15 days after insemination

	Untreated—with embryos	Treated—without embryos	Treated—with embryos	Untreated—without embryos	Treated—precocious ovulations—without embryos
No. of animals	20	10	18	7	14
Average ($cm.^2 \times 210^2$)	0.775	0.833	0.844	0.938	1.218
Duncan's M.R.T.[a] 1%					
5%					

[a] Treatments not underscored by a continuous line are significantly different (3).

TABLE 3

Average cross-sectional endometrial areas in normal and oxytocin-treated heifers 15 days after insemination

Group	Untreated—without embryos	Treated—with embryos	Untreated—with embryos	Treated—without embryos	Treated—precocious ovulations—without embryos
No. of animals	3	17	18	9	11
Average area ($cm.^2$)	0.480	0.505	0.517	0.546	0.857
Duncan's M.R.T. (3) 1% and 5%					

TABLE 4

Average endometrial surface epithelial cell heights[a] in normal and oxytocin-treated heifers 15 days after insemination

Group	Untreated—without embryos	Treated—with embryos	Untreated—with embryos	Treated—without embryos	Treated—precocious ovulations—without embryos
No. of animals	6	18	19	9	14
Average cell height ($\mu \times 100$)	3.493	3.561	3.261	3.459	3.231
Duncan's M.R.T. (3) 5%					

[a] Fifty cell heights were measured for each heifer.

The oxytocin-treated heifers which had precocious ovulations had larger endometrial glands and thicker endometria than either the treated or the untreated heifers having embryos. The endometrial surface epithelial cell height was also significantly lowered in the treated heifers which had precocious ovulations. Significant differences were not found in any of these measurements among the four other groups of heifers.

Sections from the ovarian end of the oviducts from heifers having embryos, both treated and untreated, were characterized by numerous nuclei extruded into the lumen from the epithelial cells. Many protoplasmic projections were also noted. In marked contrast, very few extruded nuclei and protoplasmic projections were found in the oviducts of the treated heifers that ovulated precociously. The epithelial cells were generally taller and narrower and had more elongated nuclei than similar cells in the oviducts of the heifers having embryos. The cilia were also especially prominent in sections of the oviducts of the heifers which ovulated precociously.

The thyroid glands. No differences were found among the thyroid epithelial cell heights of the heifers in the various groups. Average cell heights of 10.00, 11.72, 10.14, 10.41, and 10.30 μ were obtained for the untreated heifers with embryos, the untreated heifers without embryos, the treated heifers with embryos, the treated heifers without embryos, and the treated heifers that had a precocious ovulation, respectively. The slightly higher average value for the untreated heifers without embryos was due to one heifer having a definitely hyperplastic gland. The average acinar cell height for heifers having cystic corpora lutea was 10.64 μ, a figure comparable to that obtained for the other groups.

The pituitary glands. Results obtained by study of the PAS stained anterior pituitary glands from the heifers in each group are summarized in Table 5, from which it may be seen that the delta cells were moderately to heavily granulated in the glands from all heifers, both treated and untreated, that furnished embryos. These same cells were almost completely degranulated in the untreated heifers that did not have embryos. The degree of granulation seen in these cells

TABLE 5

Degree of pituitary delta cell granulation in normal and oxytocin-treated heifers 15 days after insemination

Treatment group	No. of heifers	Degree of granulation of adenohypophysial delta cells
Untreated—with embryos	20	Moderate to heavy granulation
Treated—with embryos	18	Moderate to heavy granulation
Untreated—without embryos	7	Almost complete degranulation
Treated—without embryos	9	Slightly granulated or degranulated
Treated—without embryos—precocious ovulations	15	(a) Returned to estrus, Days 7-8—degranulated (b) Returned to estrus, Days 9-12—moderately granulated (c) Returned to estrus, Days 13-15—degranulated

in pituitaries from the treated heifers that ovulated precociously varied somewhat, depending upon when they returned to estrus during the 15-day treatment period.

The mammary glands. An increase in mammary gland size was noted to occur during the oxytocin treatment in ten heifers, four of which also developed cystic corpora lutea. In most cases, fluid resembling milk accumulated in the udder. Histological sections of these glands revealed the presence of secretory tissue. No similar mammary gland stimulation was noted in the untreated animals.

DISCUSSION

The results provided little evidence to support the idea that the rate of embryo development at this very early stage is proportional to the rate of progesterone secretion, insofar as it is represented by the level of progesterone in the corpus luteum. As previously stated, the results rather suggest that normal embryo development occurs when the level of progesterone production exceeds some threshold level represented by approximately 100 μg. of luteal progesterone. Normal embryos at the same stage of development were recovered from heifers having as little as 108 μg. and as much as 883 μg. of total luteal progesterone.

No significant differences were found in either embryo lengths or embryonic disc lengths between the treated and untreated animals. Further, neither embryo lengths nor disc lengths were significantly correlated with total progesterone levels in the corpora lutea.

Aside from the marked inhibition of the corpora lutea in the treated animals that returned to estrus, the most interesting finding in this connection is the relatively high incidence of cystic corpora lutea in the treated animals. It has been previously suggested that factors which irritate, dilate, or cause abnormal uterine contractions can cause cystic corpora lutea. These data show that the incidence of cystic corpora lutea is indeed increased by oxytocin injections, that cystic corpora produced in this way usually contain little progesterone, and that they are not usually associated with live embryos. On the other hand, the two cystic corpora lutea obtained from heifers with embryos illustrate that some cystic corpora lutea contain sufficient normal luteal cells to produce the minimal amount of progesterone necessary to maintain an embryo to the 15th day. In the majority of cases, however, it may be expected that cystic corpora lutea will be associated with early embryonic death. In general, uteri from animals whose corpora lutea were inhibited and which contained little progesterone, had larger endometrial cross-sectional areas, larger endometrial glands, and reduced endometrial cell heights. These differences were most noticeable in the uteri of treated heifers which had precocious ovulations and are indicative of relatively higher levels of estrogen and lower levels of progesterone in these animals. The endometrial glands in this group most nearly resembled those described by Asdell (1) for the eighth day of the normal estrous cycle.

The differences noted in the oviducts of the heifers with embryos and those which had no embryos and ovulated before the 15th day are also explainable on

the basis of relative levels of progesterone and estrogen. Extruded nuclei are normally most numerous at about the ninth day of the cycle in the cow when progesterone is being secreted at a high level, an observation which is in general agreement with the high incidence of this phenomenon observed in the oviducts of all of the heifers with embryos and the relatively high levels of progesterone found in their corpora lutea. The marked decrease in the number of extruded nuclei and protoplasmic projections, and the taller epithelial cells seen in the oviducts of the precociously ovulating heifers, may reflect the lowered progesterone production as well as an elevated estrogen secretion in these heifers.

The degranulation of the delta cells of the pituitaries of the untreated heifers having no embryos is remarkable, since it occurred at a time in the estrous cycle when these cells are normally granulated. The granules normally disappear within a period of a few hours at or near the beginning of estrus and regranulation occurs during the luteal phase. These facts probably account for the degranulated condition of these cells in the pituitaries of the heifers that returned to estrus just prior to being slaughtered at the 15th day. The heifers which returned to estrus at the seventh or eighth day were probably very near to returning to estrus again at the 15th day, when they were slaughtered, accounting for the degranulated condition of the delta cells in this group. The oxytocin injections did not appear to prevent the reaccumulation of granules in the delta cells, since they were moderately granulated in the pituitaries of heifers that returned to estrus at Days 9-12 inclusive.

No adequate explanation can be given for the mammary gland stimulation observed in some of the treated heifers. It is not clear whether the response represented the initiation of secretion in the glands already partially developed or whether an actual development of secretory tissue occurred during the experiment.

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EFFECTS OF CROSSBREEDING ON REPRODUCTIVE PERFORMANCE OF DAIRY CATTLE [1, 2]

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SUMMARY

Data for this study were taken on the reproductive performance of 86 first-generation female progeny in the Dairy Cattle Crossbreeding Project of the Illinois Agricultural Experiment Station. The seven measures used were services per conception, age at first calving, interval from first service to conception, gestation length, calving interval, calving date to first heat, and calving date to first service.

For the seven measures used, estimates of the effect of system of mating were consistent, small, and nonsignificant. This evidence supports the concept of the relative unimportance of nonadditive gene action in effecting variation in the measures of reproductive performance used in this study.

Progress in dairy cattle breeding has been impeded by the relatively long generation interval. It is feasible that any condition capable of altering significantly the efficiency of reproduction should correspondingly affect the possible rate of progress from breeding.

Reproductive performance is measured by factors that relate directly or indirectly to reproduction. Only a small portion of the variation within, and among, these factors in reproductive performance can be attributed to genetic variation, as is indicated by the zero or almost zero estimates of repeatability and heritability (4, 7, 12, 16, 18) for these factors. Gestation length is the only measure of reproductive performance significantly heritable. Estimates range from 0.00 to 0.64, but the majority falls within the range of from 0.30 to 0.50, as reported by DeFries *et al.* (5). There is evidence confirming the effect of environmental factors such as age of dam (11, 14), age of bull (2, 7), season (14, 15, 17), year (22), and period of sexual rest (11, 21) on the reproductive efficiency.

Reproductive performance in dairy cattle is of paramount importance. To maintain efficient production, it is necessary that cows reproduce regularly. It has been reported that lowered breeding efficiency may be associated with high production (1, 10, 13) and, contradictorily, that there is little relationship between production and breeding efficiency (3, 4, 8, 19, 20).

The object of this study is to compare purebred and crossbred offspring, to determine the effect of system of mating on reproductive performance.

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[1] Data for this study were taken from the Dairy Cattle Crossbreeding Project in progress at the Illinois Agricultural Experimental Station. This is a cooperative project between the Dairy Cattle Research Branch of the ARS, USDA, and the Department of Dairy Science of the University of Illinois, and is a contributing project to Regional Project NC-2, The Improvement of Dairy Cattle Through Breeding.

[2] Data in this paper are from a thesis submitted by the senior author to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Master of Science, August, 1960.

SOURCE AND DESCRIPTION OF DATA

Data for this study were taken on the reproductive performance on first-generation female progeny in the Dairy Cattle Crossbreeding Project in progress at the Illinois Agricultural Experimental Station.

Females included in the study were first-generation purebred Holsteins, purebred Guernseys, and reciprocal Guernsey-Holstein crosses. The purebred Holstein and Guernsey foundation cows were mated alternately to the Holstein and Guernsey bulls. Eighty-six (86) of their female progeny were included in the study and they were sired by four sires, two Holsteins and two Guernseys. The number of daughters per sire in each group ranged from seven to 14, with a mean of 10.75. The numbers in the respective groups are presented in Table 1.

Each offspring should have received a random sample of the parental genetic material and, because crossbreds and purebreds received samples of the same genes, purebreds and crossbreds were comparable without including bias.

The seven measures of reproductive performances used in this investigation were services per conception (over four lactations), age at first calving, days from first service to conception (over first and second lactation), length of gestation interval (over four lactations), length of calving interval (over four lactations), days from calving date to first heat (over four lactations), and days from calving date to first service (over four lactations).

Services per conception were based on successful pregnancy. In the case of an abortion, or other abnormal conditions, the gestation length, age at calving, and calving interval were appropriately excluded.

There was no culling among the animals except in the case of sterile individuals. An attempt was made to give all females equal and unbiased treatment; therefore, it is reasonable to assume that the data provided unbiased information on the comparisons made.

ANALYSIS OF DATA

The object of the analysis of the data was to determine the effect of crossbreeding on reproductive performance. Variation in reproductive performance may be due to other variables; therefore, an attempt was made to evaluate the effect of other variables inherent in the data.

Data were transferred from the permanent records and grouped by sires in subclasses, according to the breed of dam and by lactations. The analyses consisted of orthogonal comparisons; thus, the means of opposing effects for each measure of reproductive performance by lactation were compared and the differences were tested for statistical significance by the use of a "t"-test.

TABLE 1

Number of animals in the subgroups

	Guernsey sires		Holstein sires	
	G_1	G_2	H_1	H_2
Guernsey dams	7	9	7	14
Holstein dams	13	10	12	14

The respective variances were computed on a within lactation and subclass basis. The numbers of animals in the subgroups were unequal; therefore, it was necessary to use an inverse matrix in analysis of the data, to provide unbiased estimates of the variances of the different effects.

The following linear mathematical model was assumed to represent the data to be analyzed:

$$Y_{ijkl} = \mu + a_i + b_{ij} + c_k + ac_{ik} + bc_{ijk} + e_{ijkl}$$

in which Y_{ijkl} is the l^{th} observation in the progeny group produced when the j^{th} sire of the i^{th} breed is mated to the k^{th} breed of dam, μ is the sample mean, a_i is the effect of the i^{th} breed of sire, b_{ij} is the effect of j^{th} sire of the i^{th} breed, c_k is the effect of the k^{th} breed of dam, ac_{ik} is the effect of mating the i^{th} breed of sire to the k^{th} breed of dam, bc_{ijk} is the effect of mating the j^{th} sire of the i^{th} breed to the k^{th} breed of dam, and e_{ijkl} is the random error associated with the l^{th} observation of the ijk^{th} progeny group. All the variables were assumed to be fixed except e_{ijkl}, which was assumed to be a random variable with a mean of zero and a variance of σ^2_e.

Means of the subclasses for each measure of reproductive performance were arranged in tables by lactations. The various orthogonal comparisons for these means were derived and the means were compared to determine the effects of breed of sire, breed of dam, system of mating, sires within breeds, and sires within breeds by breed of dam. These effects (D_m) were tested for statistical significance by the use of a "t"-test in which:

$$t = \frac{D_i}{(\sigma^2_e c^{ij})^{1/2}}$$

where D_i is the quantity appearing in the difference column and defined above, c^{ij} is the diagonal element of the inverse matrix for the measure of reproductive performance in a specified lactation, and σ^2_e is the within subclass variance associated with the particular effect being tested in a specific lactation. The value of c^{ij} differed within a lactation, depending on whether four or eight subclasses means were being compared. This method of analysis has been shown in detail by Harvey (9).

RESULTS

From the data collected, and the method of analysis, it is convenient in discussing the results to consider separately the measures used and the importance of the factors effecting variation within these measures.

Obviously, these measures were altered by other factors, but it is reasonable to assume that such factors were randomly distributed and would not be confounded with any of the controlled variables. The experiment covers a rather extensive period of time and, in addition, observations were taken on as many as four lactations, where available, for each animal in the study. This should effectively cancel out, for example, the results from a poor inseminator breeding the animal and causing the animal to require more services per conception, which would correspondingly increase, where appropriate, the age at calving, the calving interval, and the days from first service to conception.

Services per conception. For all the breed groups, the mean number of services required for conception was highest in the first lactation. This suggests that first-calf heifers do not conceive as readily as older animals. Data in Table 2 indicate that the differences in the number of services required for conception by the purebred and crossbred animals were not statistically significant. The differences were small and fluctuating, and the fluctuation of these differences may indicate a random effect. The observations in this study are consistent with other reports, in which estimates of repeatability and heritability of fertility measured by the number of services per conception were approximately zero (7, 12). This indicates that fertility is not determined by additive or nonadditive gene action; therefore, the crossbred and purebred progeny should not show differences for this measure. Results in Table 2 suggest that the effect of breed of sire on the number of services required for conception was not consistent. The possible conclusions from the significant differences observed are conflicting. The effect of service sire is partially confounded with the effect of breed of sire, because approximately three-quarters of the animals in either of the groups are descendants of sires from the same breed. Until the effect of service sire is removed, a meaningful interpretation of this difference is not possible. The mean of the progeny of the Guernsey dams was consistently larger than the mean of the progeny of the Holstein dams. The differences were all small and nonsignificant. Estimates of the effect of sires within breed and the interaction of sires within breeds with breed of dam gave small and nonsignificant differences.

TABLE 2

Estimates of the effects of the variables on services per conception[a]

Effect	Lactation			
	1st	2nd	3rd	4th
$\bar{X}$	4.20	2.10	2.00	2.00
B. S.	−0.26	0.02	−0.47*	0.42*
B. D.	−0.35	−0.03	−0.07	−0.08
S. M.	0.14	−0.21	0.06	−0.05
S. E.	0.23	0.19	0.21	0.18
D. F.	78	72	62	43
H. S.	0.00	−0.18	−0.10	−0.26
H. S. × B. D.	−0.09	0.09	−0.05	0.16
S. E.	0.31	0.26	0.26	0.26
D. F.	43	41	37	21
G. S.	0.15	0.05	−0.14	0.03
G. S. × B. D.	0.04	0.01	0.05	−0.03
S. E.	0.33	0.29	0.32	0.26
D. F.	35	31	25	22

* ($P < 0.05$).

[a] Values expressed as the number of services required for successful conception.

$\bar{X}$ = Unweighted mean of the eight subclass means. B. S. = Mean of the progeny of Holstein sires minus the mean of the progeny of Guernsey sires (HS − GS). B. D. = Mean of the progeny of Holstein dams minus the mean of the progeny of Guernsey dams (HD − GD). S. M. = Mean of purebreds minus the mean of crossbreds, (PB − CB). H. S. = Mean of the progeny of Holstein Sire One minus that of Holstein Sire Two ($H_1 - H_2$). H. S. × B. D. = ($H_1H - H_1G$) − ($H_2H - H_2G$). G. S. = Mean of the progeny of Guernsey Sire One minus the mean of the progeny of Guernsey Sire Two ($G_1 - G_2$). G. S. × B. D. = ($G_1H - G_1G$) − ($G_2H - G_2G$). S. E. = The standard error associated with the differences. D. F. = Degrees of freedom on which the standard errors are based.

Age at first calving. Differences between the mean age at first calving for the progeny of the Holstein sires and the progeny of the Guernsey sires were negligible. The mean age of the progeny of the Guernsey dams was 6.0 days greater than the mean age of the progeny of the Holstein dams, and the crossbred animals calved 4.4 days earlier than the purebreds. None of the differences was significant. In this project, all the females were bred for the first time on the first heat after 15 mo. of age and then on succeeding heat periods until conception. Results presented in Table 3 indicate operation of the project has been successful in having the various breed groups calve at approximately the same age.

Days from first service to conception. Differences between the mean interval for the crossbred and purebred animals were not consistent. The estimates were small and nonsignificant. This is in agreement with previous work (7), where estimates of heritability and repeatability of this measure yield values that are approximately zero; therefore, one would not expect to find genetically effected differences. The mean of the progeny of the Holstein sires exceeded the mean of the progeny of the Guernsey sires by a maximum of 8.6 days, and this difference was not significant, as is indicated by results in Table 4. The effect of breed of dam, sires within breed, and interaction of sires within breeds with breed of dam did not significantly affect the interval from first service to conception.

Gestation length. Differences between the mean gestation length of the purebred and crossbred progeny were not consistent. The values were small, with a maximum difference of 1.58 days in the fourth lactation. As presented in Table 5, all the differences for system of mating were nonsignificant. From the data in this study, cross-breeding has not altered significantly the gestation length of dairy cattle. This is in agreement with another study (5), in which it appears as if nonadditive hereditary effects are of minor importance in influencing the length of the gestation period. Data presented in Table 5 indicate probable breed difference in the mean gestation length. This has been stated by DeFries *et al.* (5). The progeny of the Guernsey sires had a mean gestation

TABLE 3

Estimates of the effects of the variables on age at first calving[a]

Effect	
$\bar{X}$	876.0
B. S.	−0.2
B. D.	−5.8
S. M.	4.4
S. E.	8.6
D. F.	75
H. S.	−4.4
H. S. × B. D.	−7.5
S. E.	11.9
D. F.	40
G. S.	0.7
G. S. × B. D.	0.7
S. E.	12.5
D. F.	35

[a] Values expressed in days.

TABLE 4
Estimates of the effects of the variables on the interval from first service to conception [a]

Effect	Lactation	
	1st	2nd
$\bar{x}$	120.0	41.0
B. S.	−0.1	8.6
B. D.	−10.3	1.4
S. M.	9.9	−4.8
S. E.	8.7	7.9
D. F.	78	72
H. S.	5.6	−11.0
H. S. × B. D.	−5.9	2.4
S. E.	11.8	10.1
D. F.	43	41
G. S.	10.2	0.1
G. S. × B. D.	−5.8	−0.6
S. E.	12.7	11.7
D. F.	35	31

[a] Values expressed in days.

TABLE 5
Estimates of the effects of the variables on gestation length [a]

Effect	Lactation			
	1st	2nd	3rd	4th
$\bar{x}$	281.0	282.0	283.0	280.0
B. S.	−1.10	−1.52*	−2.41**	−0.57
B. D.	−1.50*	−1.45*	−1.21	0.57
S. M.	0.08	0.69	0.12	−1.58
S. E.	0.65	0.65	0.87	1.59
D. F.	75	70	62	46
H. S.	−0.12	0.54	−0.46	0.13
H. S. × B. D.	−0.60	−0.53	−0.21	−0.98
S. E.	0.89	0.87	1.11	2.02
D. F.	40	40	36	24
G. S.	−0.16	0.12	−0.14	−2.55
G. S. × B. D.	0.04	−0.05	0.24	1.64
S. E.	0.94	0.96	1.34	2.47
D. F.	35	30	26	20

* ($P < 0.05$).
** ($P < 0.01$).
[a] Values expressed in days.

length consistently longer than the mean gestation length of the offspring of the Holstein sires. Differences in the second and third lactation as shown in Table 5 were significant at the 0.05 and 0.01 levels, respectively. It is not possible to draw conclusions from the significant differences, because of partial confounding of the effect of service sire with the effect of breed of sire. The mean gestation length of the progeny of the Guernsey dams was generally longer than the mean gestation length of the offspring of the Holstein dams. The differences for the first and second lactation were significant at the 0.05 level, but again this is confounded with the effect of service sire. Estimates of the effect of sires within breed were small. Estimates for the effect of interaction of sires within breeds with breed of dam were negligible and all differences were nonsignificant.

Calving interval. Differences between the mean calving interval of the

purebred and crossbred animals fluctuated, but these differences were not significant. Calving interval is determined by the gestation length and the interval from parturition to conception. There is not a wide variation in the average gestation length; therefore, one expects that possible alterations in the length of the calving interval will be effected by alterations in the interval from calving to conception. The available information indicates that the variation in the length of this interval is not determined directly by gene action; therefore, the observations in this study are consistent with such a concept. The differences between the mean calving interval of the progeny of the Holstein sires and the progeny of the Guernsey sires were not consistent. Results are presented in Table 6. In the first and third lactations, the mean of the progeny of the Holstein sires was larger and the difference of 15.9 days in the third lactation was significant at the 0.05 level. This estimate, being partially confounded with the effect of service sire, can not be exactly interpreted. Estimates of the effect of breed of dam, sires within breed, and the interaction of sires within breeds with breed of dam were all nonsignificant.

Calving date to first heat. Comparison of the mean number of days for the purebred and crossbred animals yielded small differences that fluctuated. All the differences were nonsignificant. Results presented in Table 7 indicate that the mean number of days for the progeny of the Holstein sires was consistently longer than the mean interval for the progeny of the Guernsey sires. Differences of 8.7 and 8.8 days for the first and third lactations were significant at the 0.05 and 0.01 levels, respectively. This suggests possible breed differences in the length of this interval. The estimated effects of breed of dam, sires within breed, and the interaction of sires within breeds with breed of dam were nonsignificant.

Calving date to first service. In this project, an attempt was made to control the length of this interval. All females were rebred on the first heat, 55 days after calving, and results presented in Table 8 indicate this was accomplished. Estimates of the effects of breed of sire, breed of dam, sires within

TABLE 6

Estimates of the effects of the variables on calving interval[a]

Effect	Lactation			
	1st	2nd	3rd	4th
$\bar{x}$	400.0	396.0	378.0	372.0
B. S.	8.7	−17.6	15.9*	−8.6
B. D.	3.6	4.4	6.0	2.6
S. M.	−4.7	−7.4	0.6	2.5
S. E.	8.9	9.7	6.7	12.0
D. F.	70	62	47	25
H. S.	−17.2	−7.1	−0.0	12.7
H. S. × B. D.	5.3	−2.6	4.8	−5.6
S. E.	12.1	12.4	8.8	17.1
D. F.	40	36	26	14
G. S.	−1.7	−6.9	1.3	15.7
G. S. × B. D.	0.8	−6.7	−1.5	−5.1
S. E.	13.2	15.0	10.2	16.7
D. F.	30	26	21	11

* ($P < 0.05$).

[a] Values expressed in days.

TABLE 7

Estimates of the effects of the variables on the interval from calving to first heat [a]

Effect	Lactation			
	1st	2nd	3rd	4th
$\bar{X}$	48.0	40.0	36.0	44.0
B. S.	8.7*	5.4	8.8**	6.6
B. D.	−1.0	−2.3	−0.0	−2.6
S. M.	−2.4	0.9	1.0	−1.6
S. E.	3.3	3.0	3.0	7.5
D. F.	74	71	40	5
H. S.	−0.9	−1.0	−2.9	2.4
H. S. × B. D.	0.9	−1.2	−0.4	−0.7
S. E.	4.4	4.0	4.2	10.2
D. F.	42	40	21	3
G. S.	0.9	1.1	−3.3	0.3
G. S. × B. D.	−1.0	3.3	2.0	2.0
S. E.	4.9	4.4	4.3	11.0
D.F.	32	31	19	2

* ($P < 0.05$).
** ($P < 0.01$).
[a] Values expressed in days.

TABLE 8

Estimates of the effects of the variables on the interval from calving to first service [a]

Effect	Lactation			
	1st	2nd	3rd	4th
$\bar{X}$	78.0	75.0	73.0	64.0
B. S.	4.8	3.7	−1.1	−12.8
B. D.	−1.1	2.0	2.7	− 9.2
S. M.	−3.4	−3.2	−1.6	2.9
S. E.	3.3	2.8	5.6	6.4
D. F.	74	69	49	25
H. S.	−4.9	−4.9	1.8	2.2
H. S. × B. D.	−0.6	−1.3	2.1	3.1
S. E.	4.4	3.7	7.5	7.7
D. F.	42	40	26	14
G. S.	−1.2	−2.3	−0.6	6.0
G. S. × B. D.	2.1	−1.5	−8.2	−6.9
S. E.	5.0	4.3	8.4	10.3
D. F.	32	29	23	11

[a] Values expressed in days.

breed, the interactions, sires within breeds by breed of dam, and system of mating were all nonsignificant.

Conception at first service. Analysis of the results on conception at first service, as presented in Table 9, indicates that all the effects tested were nonsignificant.

DISCUSSION

The data in this study have yielded consistent results. Where the differences were significant, it was not possible to draw conclusions, because the effect of service sires was partially confounded with the effect of breed of sire, and the effect of breed of dam. The effect of service sire was not confounded with the system of mating and there were no significant differences between the purebred and crossbred animals for any of the seven measures used. This evidence is in agreement with the concept that the seven measures of repro-

TABLE 9

Estimates of the effects of the variables on the conception at first service [a]

Effect	Lactation			
	1st	2nd	3rd	4th
$\bar{x}$	0.095	0.488	0.571	0.461
B. S.	−0.018	0.032	0.103	−0.130
B. D.	0.004	0.024	0.051	0.009
S. M.	0.002	−0.003	−0.002	0.025
S. E.	0.034	0.058	0.063	0.078
D. F.	78	72	62	43
H. S.	0.003	0.055	0.066	0.053
H. S. × B. D.	0.039	−0.053	0.022	−0.004
S. E.	0.046	0.078	0.079	0.109
D. F.	43	41	37	21
G. S.	−0.057	−0.006	0.040	−0.031
G. S. × B. D.	−0.001	0.065	−0.001	0.031
S. E.	0.050	0.087	0.098	0.111
D. F.	35	31	25	22

[a] A value of one was given to animals conceiving on the first service and a value of zero to animals failing to conceive on the first service. Values in the table are the differences between the means after weighting with orthogonal coefficients.

ductive performance included in this study are altered by factors not governed by nonadditive gene action.

In dairying, efficient production is dependent on efficient reproduction; therefore, it is reasonable to infer that if the cattle breeder is attempting to improve the measures of reproductive efficiency included in this study, he will not be successful by cross-breeding to utilize nonadditive gene effects.

It was mentioned earlier that culling was not practiced, except in the case of sterile animals. A total of five animals was sterile and two of these were purebred and three were crossbreed. With these relatively small numbers, the ratios observed were acceptable and could not be interpreted as being significantly different. The sex ratio was nearly equal for the purebred and crossbred animals. Among the purebred animals, the ratio of females to males was 80 to 84 and among the crossbred animals, 91 to 96. These relationships are approximately equal and we can assume that the sex ratio is similar in crossbred and purebred animals. The occurrence of abortion was approximately 1.8% in the purebred animals and 2.7% in the crossbred animals. These frequencies are not significantly different when considered in relation to the size of the sample.

Dickinson and Touchberry (6) reported that when the livability from birth to calving age of purebred and crossbred animals is compared, a striking difference is found. Of the purebreds, 17.4% died by 2 yr. of age, whereas only 8.2% of the crossbreds and crisscross animals died. In the present study, livability was not included as a measure of reproductive efficiency. It is important to understand that results presented in this study, except Table 9, relate only to fertile animals. Data came from a selected group of animals, i.e., animals capable of conceiving, maintaining pregnancy, and successful parturition. The conclusions are applicable only to such animals. This amounts to working with a conditional distribution, in that one is concerned with the distribution of reproductive performance, given that the animals concerned conceived and calved.

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UTILIZING BOTH PART AND COMPLETE DAUGHTER RECORDS IN SIRE EVALUATION

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SUMMARY

A three-stage program for evaluating sires used in artificial insemination is proposed. The first stage consists of acceptance or rejection of sires based on the part-lactation (5-mo.) records of their daughters. The second stage utilizes both part records and complete records of other daughters. The third and final stage provides for evaluation dependent on complete records alone. Selection index equations are presented for estimating the breeding value of sires for these combinations of information. The correlations between estimated and true breeding value are given in an example which illustrates the additional knowledge available in evaluating sires when part records are utilized. For example, ten 5-mo. records have the expected correlation with true breeding value of 0.58, as opposed to 0.00 if only complete records are used; 20, 5-mo. records, a correlation of 0.69 as opposed to 0.00; 20, 5-mo. and ten complete records, 0.78 as opposed to 0.61; and ten 5-mo. and 20 complete records, 0.79 as opposed to 0.74.

The procedures used for sampling A.I. (artificial insemination) sires follow a familiar pattern. A young, untested bull is placed in service until enough cows are mated so that 40-50 daughters will eventually have records (in a central processing laboratory) available for evaluating the sire's breeding value for milk production. Then the sire is retired from service until a proof is completed.

Recent research, Madden *et al.* (5), Searle (7), and VanVleck and Henderson (9), has shown the existence of high genetic relationships between individual monthly records and complete production records, and between cumulative monthly records (especially, five or more months in length) and complete records. These results have indicated the possibility of preliminary selection of A.I. sires on the basis of part records. In nearly all instances of sampling of young sires, a sizable number of 5-mo. daughter records would be available before any or only a few daughter lactations are completed. Intuitively, it would seem reasonable that sires with high genetic merit for milk production could be selected to return to service on the basis of 5-mo. records of their daughters. Equally as intuitive would be culling of inferior sires. This reduction in generation interval could, in itself, increase the rate of genetic progress.

If the average length of time from the sample inseminations until complete daughter records are centrally processed is 50 mo., then the rate of genetic progress would be increased about 10%, if all the sire selection decisions are made on the basis of 5-mo. records, or about 5% if one-half the decisions are made on 5-mo. records, over that accomplished by selection based entirely on complete records. This result requires that the accuracy of prediction is equal in both cases. The reduction in number of sires held out of service could de-

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crease the cost of the sampling program to the A.I. center or could allow more sires to be tested with no increase in expenditure, thus increasing the possible selection intensity which could be practiced.

Decisions on those sires whose daughters are above average, but which do not definitely mark the sires as being superior with respect to genetic merit for production, could be delayed until complete records are available. The sires marked superior on the basis of part records could also be re-examined in the light of complete records.

With these intuitive ideas in mind, a three-stage sampling program for evaluating A.I. sires will now be proposed.

Stage 1 would include selection on the basis of part records alone prior to completion of any daughter lactations. In the present situation, cumulative 5-mo. records and also, alternatively, the fifth monthly test-day records will be considered. Of course, it should be noted that some sires may never be evaluated in Stage 1 of the selection system, since some of their daughters may have complete records by the time the sire is initially evaluated. Such sires would enter the sampling program at Stage 2.

Stage 2 selection would be determined by a combination of part records and complete records of daughters. This stage would use as sources of information part records of daughters not having complete records and records of other daughters having complete lactations.

Selection in Stage 3 would utilize records of daughters all of which have completed lactations.

Procedures to estimate true breeding value of sires for the preceding situations will now be developed using the selection index approach. In the development of these procedures, it will be assumed that all records have been corrected for age, season, year, and herd effects. Adjustment methods similar to those described by Henderson *et al.* (4) and Heidhues *et al.* (1) would be suitable for adjusting records for herd-year-season effects.

Stage 1. First consider selection of sires after completion of 5-mo. records by their daughters. An estimation procedure is needed such as $I = \mu_c + b(\overline{X}_p - \mu_p)$ where $\overline{X}_p$ is the part-record daughter average, b is the appropriate weighting factor, μ_c is the breed population average for complete records (in this situation the population consists of only A.I. daughters), μ_p is the breed population average for part records, and I is the index value or estimated breeding value. Bulls are ranked according to their estimated breeding value. Henderson (3) has shown that the selection index procedure will maximize the probability of correct ranking if the distribution of the records is normal.

The underlying model associated with part and complete records is defined as: $Y_{pij} = \mu_p + s_{pi} + e_{pij}$ and

$Y_{cij} = \mu_c + s_{ci} + e_{cij}$ where the p subscript refers to a part record of prescribed length and the c subscript to a complete record. An age and herd-year-season adjusted observation Y_{ij} then is made up of a population average, μ; a sire effect, s_i; and a random error effect, e_{ij}. It is further assumed that the s_{pi}, s_{ci}, e_{pij}, and e_{cij} have zero means and variances $\sigma_{s_p}^2$, $\sigma_{s_c}^2$, $\sigma_{e_p}^2$, and $\sigma_{e_c}^2$, respectively;

the covariance between s_{pi} and s_{ci} is $\sigma_{s_{cp}}$; the covariance between e_{pij} and e_{cij} is $\sigma_{e_{pc}}$; and all other covariances are zero.

Returning to the index equations, it is known that the weighting factor, b, equals the covariance between the part-record average and breeding value for complete records, divided by the variance of the part-record average. The covariance is $\sigma_{s_{cp}}$ and the variance is $\sigma_{s_p}^2 + \frac{\sigma_{e_p}^2}{n_p}$ where n_p is the number of part records in the average, $\overline{X}_p$. Thus, the index becomes

$$I = \mu_0 + \frac{\sigma_{s_{cp}}}{\sigma_{s_p}^2 + \frac{\sigma_{e_p}^2}{n_p}} \quad (\overline{X}_p - \mu_p).$$

The correlation between the index and true breeding value T is,

$$r_{TI} = (b\, \sigma_{s_{cp}}/\sigma_{s_c}^2)^{1/2}$$

$$= \left[\frac{n_p(\sigma_{s_{cp}})^2}{(n_p\sigma_{s_p}^2 + \sigma_{e_p}^2)\sigma_{s_c}^2} \right]^{1/2}$$

The number of part records required to be as accurate as a fixed number of complete records for predicting breeding value for complete lactation production is given by VanVleck and Henderson (10) and by Searle (8). If, however, one wants to hedge, or does not feel satisfied that part records can be as accurate as complete records, then the standards for return to service may be increased. A rating between the low standard for culling and the high standard for return to service use would entitle a bull to a second chance based on complete records or on more part records.

TABLE 1

Variance and covariance components used in regression equations [a]

Component	5-Mo. records	Fifth test-day records	Complete records
$\hat{\sigma}_e^2$	1,886.00	85.23	5,095.40
$\hat{\sigma}_s^2$	112.31	4.15	305.80
$\hat{\sigma}_{s_{cp}}$	174.58	35.88	305.80

[a] These components are extracted from Table 2—5-mo. records, Table 1—fifth test-day records, and Table 2—complete records of VanVleck and Henderson (10).

$\hat{\sigma}_e^2$ is the estimated residual variance component,

$\hat{\sigma}_s^2$ is the estimated sire variance component, and

$\hat{\sigma}_{s_{cp}}$ is the estimated sire covariance component between part and complete records.

The estimated values of the necessary variances and covariances to construct an estimate of b can be obtained from tables developed by VanVleck and

Henderson (10), extracted and given in Table 1. The b value for 5-mo. records is

$$b = \frac{174.58}{112.31 + \frac{1886.00}{n_p}} = \frac{1.55 n_p}{n_p + 16.79} \text{ and the correlation is}$$

$$r_{TI} = \frac{174.58}{\left[\left(112.31 + \frac{1886.00}{n_p}\right)(305.80)\right]^{1/2}}$$

$$= 9.9819 / \left[\frac{112.31 + 1886.00}{n_p}\right]^{1/2}$$

Alternatively, the b value for the fifth monthly test records is

$$b = \frac{35.88}{4.15 + \frac{85.23}{n_p}} = \frac{8.65 n_p}{n_p + 20.54} \text{ and}$$

$$r_{TI} = 2.0515 / \left[4.15 + \frac{85.23}{n_p}\right]^{1/2}.$$

Although one would expect the correlation to be higher for 5-mo. cumulative records than for fifth test-day records with a complete record, they are nearly equal. For example, when $n_p = 10$ the correlation is 0.5752 for the former and 0.5762 for the latter. These correlations do not appear high, but no matter which procedure is used each procedure is better than having no knowledge of a sire's genetic value, as would be the situation if one were forced to wait until complete records were available.

As an example, suppose that the average of the age and herd-year-season adjusted 5-mo. records of 20 daughters of an A.I. sire is 8,000 lb. of milk, the A.I. average of complete records is 10,000 lb., and the A.I. average of 5-mo. records is 7,500 lb. of milk. The predicted breeding value of the sire is then

$$I = 10{,}000 + \frac{1.55(20)}{20 + 16.79}(8{,}000 - 7{,}500) = 10{,}421 \text{ lb. of milk.}$$

Since the index value is above the population average, the sire probably would not be culled and may even be returned to service. The correlation between predicted and actual breeding value for complete records for sires with 20 available part records is 0.69, compared to 0.74 for estimation with 20 complete records.

Stage 2. As outlined previously, Stage 2 selection would make use of part records of daughters not having complete records, in combination with complete records of daughters having finished one lactation. The same model for part and whole records will be assumed as in Stage 1. Now the index will be of the form

$I = \mu_c + b_1(\bar{X}_p - \mu_p) + b_2(\bar{X}_c - \mu_c)$ where $\bar{X}_c$ is the average of n_c completed records of a sire's daughters. The normal equations to be solved in order to determine the b's are

$$b_1\left[\sigma_{s_p}^2 + \frac{\sigma_{e_p}^2}{n_p}\right] + b_2\sigma_{s_{cp}} = \sigma_{s_{cp}}$$

$$b_1\sigma_{s_{cp}} + b_2\left[\sigma_{s_c}^2 + \frac{\sigma_{e_c}^2}{n_c}\right] = \sigma_{s_c}^2.$$

Solving for the b's provides the estimators

$$b_1 = \frac{\sigma_{s_{cp}}\left[\frac{\sigma_{e_c}^2}{n_c}\right]}{\left[\sigma_{s_p}^2 + \frac{\sigma_{e_p}^2}{n_p}\right]\left[\sigma_{s_c}^2 + \frac{\sigma_{e_c}^2}{n_c}\right] - (\sigma_{s_{cp}})^2} \quad \text{and}$$

$$b_2 = \frac{\left[\sigma_{s_p}^2 + \frac{\sigma_{e_p}^2}{n_p}\right]\sigma_{s_c}^2 - (\sigma_{s_{cp}})^2}{\left[\sigma_{s_p}^2 + \frac{\sigma_{e_p}^2}{n_p}\right]\left[\sigma_{s_c}^2 + \frac{\sigma_{e_c}^2}{n_c}\right] - (\sigma_{s_{cp}})^2}.$$

Note that the denominators of the expressions for b_1 and b_2 are equal and also note that several of the terms are used more than once.

The correlation between the actual and predicted breeding value is

$$r_{TI} = \left[\frac{b_1\sigma_{s_{cp}} + b_2\sigma_{s_c}^2}{\sigma_{s_c}^2}\right]^{1/2}.$$

Again using Table 1, the estimates of b_1, b_2, and r_{TI} for the index using 5-mo. records and complete records are

$$b_1 = \frac{\left((174.58)\right)\left(\frac{5095.40}{n_c}\right)}{\left(112.31 + \frac{1886.00}{n_p}\right)\left(305.80 + \frac{5095.40}{n_c}\right) - (174.58)^2},$$

$$b_2 = \frac{\left(112.31 + \frac{1886.00}{n_p}\right)(305.80) - (174.58)^2}{\left(112.31 + \frac{1886.00}{n_p}\right)\left(305.80 + \frac{5095.40}{n_c}\right) - (174.58)^2}, \quad \text{and}$$

$$r_{TI} = \left(\frac{174.58b_1 + 305.80b_2}{305.80}\right)^{1/2}$$

The expected correlations between true breeding value and the index value when $n_p = 10$ and $n_c = 10$ will illustrate the value of the part records. For the system which uses 5-mo. and complete records $r_{TI} = 0.72$ and likewise $r_{TI} = 0.72$ for the system which uses the fifth monthly test-day records and complete records. These correlations are large relative to the $r_{TI} = 0.61$ for ten complete records alone and to the $r_{TI} = 0.74$ for 20 complete records. Thus, if the same amount of information was available for each sire—ten 5-mo. records and ten complete records—then the gain in accuracy by using all information over using only complete records is

$$\left(\frac{0.72 - 0.61}{0.61}\right)100 = 18\%.$$

Stage 3. During some period of time after the initial sample inseminations, all records of a sampled sire's daughters will be complete. This period may occur before or after additional cows are inseminated based on prior selection of the sire on the basis of part or part and complete records. Stage 3 of the sampling program utilizes only complete records. It must be noted that selection decisions may have been made earlier during Stage 1 or Stage 2. Sires culled can not be redeemed, but sires returned to service as a result of Stage 1 or Stage 2 decisions can be withdrawn from service if more information becomes available which indicates they are actually substandard. Or, these sires may be used more heavily if the additional information shows them to be highly superior.

The same model and assumptions are again considered as in Stages 1 and 2. The index equation is similar in form to the one of Stage 1 where now $I = \mu_c + b(\overline{X}_c - \mu_c)$. The weighting factor, b, is, however, $\dfrac{\sigma_{s_c}^2}{\sigma_{s_c}^2 + \dfrac{\sigma_{e_c}^2}{n_c}}$. This factor can be rewritten as $\dfrac{n_c}{n_c + \dfrac{\sigma_{e_c}^2}{\sigma_{s_c}^2}}$ which has been reported to be $\dfrac{n}{n+12}$, $\dfrac{n}{n+18}$, and $\dfrac{n}{n+17}$ by various investigators, Henderson *et al.* (4), Robertson and Rendel (6), and Heidhues *et al.* (2), respectively. In this study $b = \dfrac{n_c}{n_c + 16.66}$ and $r_{TI} = \left(\dfrac{n_c}{n_c + 16.66}\right)^{1/2}$ when using only complete records.

Now that the three general stages of the selection program have been described, let us look at an example of how such a procedure will be helpful. To simplify the problem, it will be assumed that the same number of daughter records are available from each sire at each stage of selection. Suppose 30 daughter records are to become available from each sire equally spaced in time over a 1-yr. period, i.e., the beginnings of the reported records are equally spaced over a 1-yr. period, and further suppose that sire evaluations are made at 4-mo. intervals (three times per year). Then, for the first evaluation ten

5-mo. records will be available (Stage 1a), for the second evaluation 20, 5-mo. records will be available (Stage 1b), for the third evaluation 20, 5-mo. records and ten complete records (Stage 2a), for the fourth evaluation ten 5-mo. records and 20 complete records (Stage 2b), and for the fifth and final evaluation 30 complete records (Stage 3). A look at the expected correlations between the index values and true breeding value for the five possible evaluations may help to determine relative points of selection on the breeding value scale. Table 2 presents the expected correlations corresponding to the five possible evaluations, as well as the expected correlations when using only complete records at the same stages. It is easily apparent, from comparing the column labeled r_{TI} and the column labeled r_{TI} using only complete records, that the increase in accuracy of prediction is substantial if part records are used, particularly when no complete records are available.

TABLE 2

Expected correlations between predicted and actual breeding value for various combinations of 5-mo. and complete records

Stage of evaluation	Number of records		r_{TI}	r_{TI} Using only complete records
	5-Mo.	Complete		
1a	10	0	0.58	0.00
1b	20	0	0.69	0.00
2a	20	10	0.78	0.61
2b	10	20	0.79	0.74
3	0	30	0.80	0.80

A logical way of utilizing the five selection steps would be similar to the situation described in Figure 1. Suppose that the first vertical line in Figure 1 refers to possible values of breeding value expressed as deviations from the population average. The possible values as expressed on the line range from negative values on the bottom to positive values at the top—the population average would be represented at the zero point midway from top to bottom on the line.

At Stage 1a the accuracy of prediction is lower than for the other stages, so one would want to hedge in the selection decisions. The standard for return to service would be set at a high level and the standard for culling would be set at a low level. Sires with index values falling between these levels would be considered at the next selection stage. With a higher accuracy of prediction at Stage 1b, the standards would be moved closer to the center of the scale. And, as the accuracy of prediction improves the standards would continue to be moved toward the population average. Naturally, the animals that are left in the questionable group are a problem. After the final stage of selection still not enough information may be available in order to assign them to the slaughterhouse, or to the bull stud for service. If more records will not become available, then it would seem the safest alternative to agree on a final decision either to cull or to retain these bulls, depending on the requirements of the bull stud.

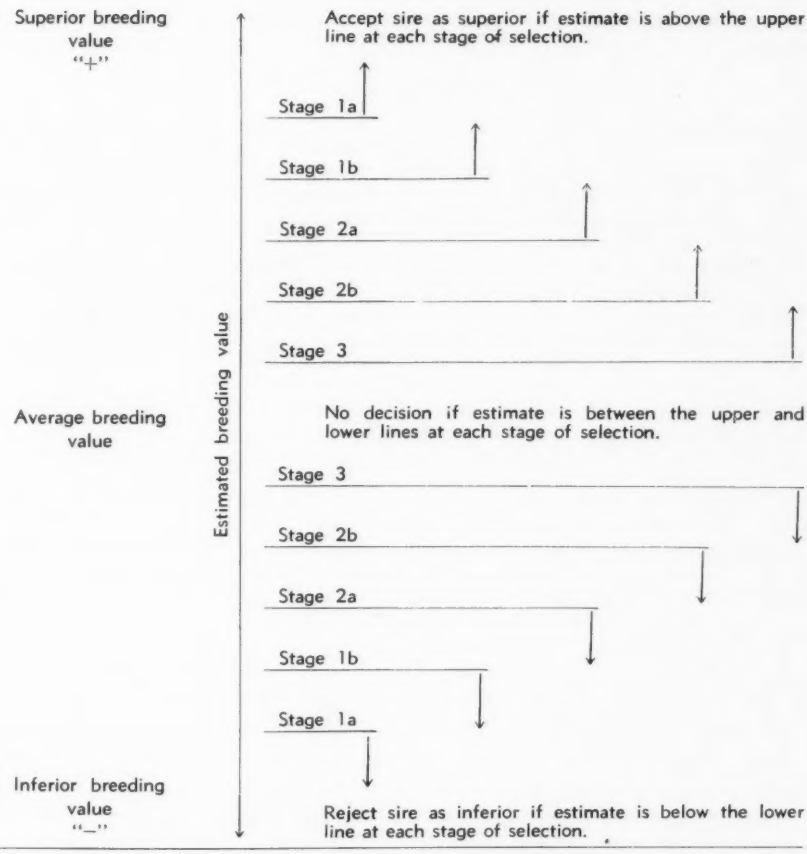
Superior breeding value "+"
Accept sire as superior if estimate is above the upper line at each stage of selection.
Stage 1a
Stage 1b
Stage 2a
Stage 2b
Stage 3
Estimated breeding value
Average breeding value
No decision if estimate is between the upper and lower lines at each stage of selection.
Stage 3
Stage 2b
Stage 2a
Stage 1b
Stage 1a
Inferior breeding value "−"
Reject sire as inferior if estimate is below the lower line at each stage of selection.

FIG. 1. Example of the use of different selection or culling levels, depending on the correlation between predicted and actual breeding value for various numbers of part and complete records.

CONCLUSIONS

Part lactation records provide valuable additional information when evaluating A.I. sires. These part records can be used by themselves or used together with complete records to increase the accuracy of predicting breeding value for total lactation yield in selection programs and thus increase progress for genetic merit. Selection on the basis of part records can reduce significantly the generation interval and thus substantially increase genetic progress, especially if enough part records are available to afford high accuracy of prediction. The use of the three logical steps which occur in sire-proving systems—part records, part and complete records, and complete records—can materially reduce costs of testing, increase the rate of genetic progress through a shorter average generation

interval and increased accuracy of prediction, or provide a combination of these things.

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DIURNAL PATTERNS OF BOVINE BODY TEMPERATURE

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SUMMARY

Patterns of diurnal body temperature variation were studied in dairy cattle. Classification of 102 continuously recorded daily temperature curves revealed the following features: Sixty-seven per cent showed two distinct daily elevations in temperature, 23% showed only one, 7% were polyphasic, showing three or more sustained rises, and 3% were aphasic. The incidence of these characteristic patterns was similar in normal, ovariectomized, and pregnant cows, and in young heifer calves. In general, temperatures were elevated in the mid-morning and afternoon and were low during the evening and early morning hours. Of the several categories of cattle studied, calves had the highest temperatures, with pregnant cows, normal cows, and ovariectomized cows showing decreasingly lower temperatures in the order listed.

In studies of the effect of reproductive state on the body temperature of cows (7, 8), it was noted that a diurnal pattern of body temperature existed for most of the cows studied.

The purpose of the present investigation was to further characterize these diurnal patterns and to study the hormonal influence on body temperature of cattle in several endocrine states.

METHODS

Vaginal temperatures were measured continuously in several categories of dairy cattle. These categories were: (1) Normally cycling cows, (2) ovariectomized cows, (3) pregnant cows, and (4) young heifer calves. Body temperature was measured as outlined previously (8), by using a resistance thermometer of the thermistor bridge type and a strip chart recorder. All observations were made on animals confined to stalls of heated barns; records were not taken during hot summer or severe winter weather.

RESULTS AND DISCUSSION

A variety of diurnal patterns was found to exist among the 102 daily records obtained. When these daily temperature curves were arbitrarily classified as to general type, it was found that 67% of them described a diphasic pattern, whereas 23% were found to be monophasic and 7% polyphasic. Straight line responses, i.e. aphasic, were observed in 3% of the curves. Typical examples of these types are shown in Figure 1. The same frequency of each type of curve was found in all the categories of animals. In general, the temperature pattern exhibited by an animal was of characteristic form, and was almost exactly superimposable on those obtained on successive days.

Inspection of the daily body temperature curves (Figure 1, diphasic pattern) revealed the following general features: (a) Elevations in the morning (5-7 A.M.) and again in the afternoon (1-6 P.M.). (b) The afternoon rise was usually

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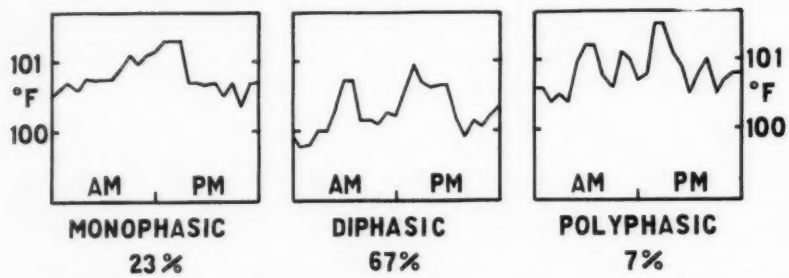

FIG. 1. Types of diurnal temperature patterns. (Curves shown are actual records transposed from the strip charts.)

higher than the morning peak. The mean time at which the highest temperature occurred was 1:50 P.M., with the normally cycling and ovariectomized cows tending to be high somewhat earlier, and the pregnant cows and the calves showing daily highs later in the afternoon. (c) Body temperature was low in the evening and early hours of the morning. The mean time at which the daily low temperature occurred was 10:25 A.M. (d) The mean daily variation of body temperature, from high to low, was 1.22° F. (range, 0.6-2.7° F.).

Table 1 shows the mean temperatures of the groups studied. Calves had the highest mean temperatures (101.6° F.), there being about one degree difference between calves and normally cycling cows (100.7° F.). The calves studied were from 24 days to 170 days of age. Even the two youngest calves, age 24 and 32 days, showed clearly diphasic daily temperature patterns, with mean temperatures higher than those of adult animals.

This finding was somewhat surprising in view of the literature on humans and birds. In these species, very young animals do not exhibit either a temperature as high as the adult or the adult cyclic pattern. Thus Kleitman, Titelbaum, and Hoffman (5) found that the adult type of diurnal temperature curve was not definitely established until the second year of life in human infants. During the first year temperature variations were noted, but no diurnal regularity was apparent. Moreover, the range of body temperature variation was slight, and the mean temperature was below that of the adult.

Lamoreux and Hutt (6), in a study of 398 chicks, found that the body

TABLE 1

Vaginal temperatures of dairy cattle

Category of dairy cattle	No. animals	No. days	Mean daily Temp. ° F.	High ° F.	Low ° F.	Variation ° F.
Normal cycling cows	4	44	100.73	101.41	100.15	1.26
Ovariectomized cows	4	31	100.62	101.32	99.96	1.36
Pregnant cows	3	6	101.02	101.60	100.42	1.18
Calves	6	21	101.62	102.18	101.18	1.00

temperature rises from 103° F. at hatching to 106° F. at seven to ten days. It appeared to take 1-2 wk. to develop the adult body temperature. If the natural longevity of the cow is taken to be 15 yr., and that of the chicken 6 yr., then our calves were in the same period of life as the chicks studied. The calf temperatures, however, were not below those of the adult but were above them.

The highest mean temperature shown by the adult cows was found to occur in the pregnant group (all in the last trimester of pregnancy). This higher temperature during pregnancy is in accord with the continued secretion of progesterone during gestation, both from the corpus luteum of pregnancy and from placental production. As has been shown previously, progesterone exerts a thermogenic influence (8). Curtailment of this progesterone influence probably accounts for the slightly lower temperatures observed in the group of ovariectomized cows (Table 1).

The extent of daily temperature variation in cycling cows and young animals (1.00 to 1.26° F.—Table 1), and the variety of the diurnal patterns observed during continuous temperature recording, suggest that it would be difficult to utilize a single, once-daily temperature determination as an indicator of ovarian activity. The diurnal variations observed in the present study are of the same order of magnitude as those which we found to occur during the temperature shift of estrus and ovulation (7). Howes, Warnick, and Hentges (3) have recently attempted to correlate once-daily vaginal temperature determinations with estrus and ovarian activity in cows. They observed characteristic vaginal temperature changes in 62 out of 74 instances where corpora lutea were detected, but a larger number of similar temperature changes were not associated with ovarian activity. Considering these results, it appears that the utility of using single daily temperature measurements as an indicator of reproductive function is rather limited for practical field use. While ovarian activity can be followed by continuous temperature recording, its usefulness is limited because of the complexity of the technique. It has some value, however, as a useful research tool in endocrine investigation.

The extent to which the biological periodism evident in these temperatures is dependent on environmental factors is not known. It is recognized that alterations in feeding habits and photoperiods frequently are accompanied by changes in the characteristic patterns of body temperature of many animals and birds (4).

Rhythmic changes based upon a 24-hr. period have been extensively reviewed in recent years (1, 2, 4). Those rhythms found to exhibit a 24-hr. cyclic pattern have been termed circadian by Halberg (2). The diurnal patterns of temperature which we observed in cattle exhibit a circadian rhythm of either diphasic, monophasic, or polyphasic character.

ACKNOWLEDGMENT

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COMPARISON OF THE ANTHRONE REAGENT AND A COPPER-REDUCTION METHOD FOR DETERMINING BLOOD SUGAR IN CALVES [1]

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SUMMARY

Protein-free filtrates from 1,500 samples of blood from 12 calves were prepared according to Folin-Wu. An aliquot of each filtrate was analyzed for blood sugar by both the anthrone reagent and the Somogyi-Nelson technique. The anthrone procedure is as follows: Pipet 1 ml. of sample filtrate, blank, or standard into a test tube and chill; add 10 ml. of ice-cold 0.05% anthrone dissolved in 72% sulfuric acid to each test tube while immersed in ice water and mix. After equalizing the temperature of the tubes in a tap-water bath, place them in a boiling water bath for 10 min.; cool in a running tap-water bath. Pour the reactants into matched spectrophotometer cuvettes and read at a wavelength of 620 mμ after aging for one-half hour in the dark. Statistical analysis indicated that the difference between the two techniques was highly significant. The anthrone values were less than 5% greater. The correlation coefficient between the results of the two methods was 0.94. The inherent error in both methods was the same, as indicated by almost identical standard deviations.

The use of anthrone (9,10-dihydro-9-ketoanthracene) in strong sulfuric acid to detect the presence of carbohydrates was introduced by Dreywood (2) in 1946. Since that time, quantitative procedures using the reagent have been developed for many classes of carbohydrates. However, for the analysis of blood sugar, relatively scant information is available (3, 6, 14).

The methods referred to above leave some doubt as to the ideal conditions for the estimation of blood sugar using the anthrone reagent. Therefore, it is the purpose of this paper to report the results of experiments to standardize the anthrone procedure for blood sugar estimation.

To test the reliability of the recommended anthrone procedure, 1,517 samples of blood from 12 calves were analyzed for their sugar content, using both the anthrone reagent and the method of Somogyi (17), with the color reagent of Nelson (12).

EXPERIMENTAL PROCEDURE

The anthrone used in these experiments was acquired from the Nutritional Biochemicals Corporation. Various percentages of sulfuric acid were prepared by adding reagent-grade sulfuric acid to a predetermined quantity of distilled water on a v/v basis.

All anthrone reagents were prepared in amber bottles by dissolving the desired amount of anthrone in the designated concentration of sulfuric acid with

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the aid of a magnetic stirrer. The reagents were aged 18-24 hr. at 3 degrees C. before use. Corrections for day-to-day variations in the reagent were made by running 25- and 100-μg. standard glucose solutions along with each group of unknowns. The standards were prepared according to Roe (14), by dissolving the desired amount of glucose in saturated benzoic acid.

The blood analyzed in these experiments was withdrawn through polyethylene catheters installed in the jugular veins of 12 dairy calves at an average age of ten days according to the technique of Ralston *et al.* (13). One-tenth milliliter of 10% potassium oxalate was added to each blood tube and evaporated to dryness for each 10 ml. of blood, to prevent coagulation. Heparin should not be used as an anticoagulant, because it reacts with anthrone. Protein-free filtrates of the blood were prepared according to Folin and Wu (4), within 5 min. after drawing, then stored in a deep-freeze until needed.

RESULTS AND DISCUSSION

The influence of the percentage of anthrone in the reagent (10, 25, 50, 75, 100 mg. %) on the anthrone-glucose color was investigated. It was concluded, in agreement with Bailey (1), that 50 mg. % anthrone dissolved in 72% sulfuric acid produced excellent results.

There appeared to be an inconsistency in the literature concerning the anthrone-glucose color. Roe (14) reported the reaction to result in a blue color, whereas Bailey (1) referred to the color as bluish-green. Since Roe (14) used 66% sulfuric acid and Bailey (1) used 72% in making their respective reagents, it was suspected that the color effect was due to the different concentrations of sulfuric acid employed. To verify the suspicion, 100 ml. of varying concentrations of sulfuric acid were used to dissolve 50-mg. amounts of anthrone. Into test tubes containing 1 ml. of glucose standard (100 μg/ml), 10 ml. of the various anthrone reagents were added. After heating in a boiling water bath, the following colors were observed to have been produced by the various anthrone-glucose reactions: 60% sulfuric acid, aqua; 65%, light blue; 70%, greenish-blue; 75%, blue-green; 80%, yellow-green; 85%, grey-green; 90%, greenish-yellow; and 95%, greyish-brown.

Figure 1 shows the absorption spectra for the colored solutions produced above. All of the anthrone-glucose reactions produced colors having a maximum optical density at 620 mμ, except those using 90 and 95% sulfuric acid, which had a maximum between 560 and 580 mμ. This finding with 90 and 95% sulfuric acid was in apparent conflict with previous reports (2). The discrepancy probably can be explained by considering the difference in the ratio of reagent to sample used by others (3) and the present workers. Others who used 95% sulfuric acid in their reagents had a ratio of reagent to sample of 2:1 (3) or 3:1 (9), which would result in a final acid concentration varying from approximately 66 to 75%, respectively. In the present work, the final acid concentration with 1 ml. of sample and 10 ml. of anthrone reagent containing 90 or 95% sulfuric acid was 82 or 86%, respectively. Thus, it appears that the concentration of the sulfuric acid after the addition of the sample is the critical point.

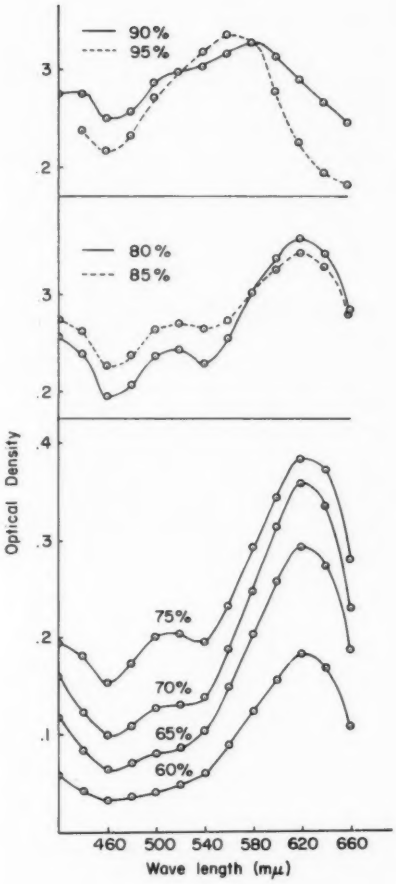

FIG. 1. The influence of various anthrone reagents, made by dissolving 50-mg. quantities of anthrone in 100 ml. of the various concentrations of sulfuric acid, on the absorption spectra of the colored solutions produced by adding 10 ml. of each reagent to 1 ml. of glucose solution (100 μg/ml). The solutions were read in a Beckman DU spectrophotometer, using matched 10-mm. silica cells.

If it exceeds 82%, the color produced by the anthrone-glucose reaction no longer has an absorption maximum at 620 mμ. The reason for this change in absorption maximum is not clear at this time.

The final sulfuric acid concentration after the addition of the reagent to the sample is important from another aspect; it may not be sufficiently high to retain the anthrone in solution. We found that 60% sulfuric acid dissolved 0.05% anthrone only after prolonged stirring. If 1 ml. of sample is mixed with 10 ml. of this reagent, the final acid concentration would be approximately 55%. This appears to be near the lower limit for 0.05% anthrone solubility. Therefore, if samples larger than 1 ml. are to be used, more concentrated sulfuric acid, or less anthrone, is recommended for the preparation of the reagent.

The relationship of the glucose concentration to the optical density of the anthrone-glucose color has been previously shown to be linear (1, 14).

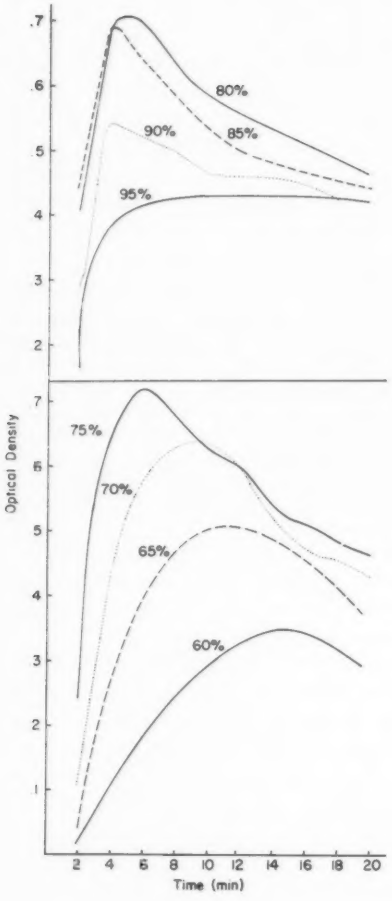

FIG. 2. The influence of the sulfuric acid concentration of the 0.05% anthrone reagent on the optical density of the anthrone-glucose solutions heated in a boiling-water bath for various lengths of time. Ten milliliters of the respective reagents were added to 1 ml. of glucose solution (100 μg/ml). The solutions were read in a Coleman Universal Model 14 spectrophotometer, using 19- by 105-mm. matched cuvettes, at a wavelength of 620 mμ.

The optimum heating time for the reaction between a carbohydrate and the anthrone reagent depends upon the nature of the carbohydrate, the concentration of the sulfuric acid, and the temperature of the incubating bath. For glucose, the heating time at 100 degrees C. decreases as the sulfuric acid concentration increases (Figure 2). A longer heating time is required if the temperature of the incubating bath is decreased.

The control of heat production before the heating of the anthrone-glucose reactants is an important feature of the procedure. Effective control can be achieved by using a lower percentage of sulfuric acid which has already lost heat during its solvation process, and by supercooling the reactants before and after mixing, by immersion in a sodium chloride crushed ice slurry.

The stability of the anthrone-glucose color is a matter of controversy. Scott and Melvin (15) recommended reading the anthrone-glucose colors immediately after cooling. At the other extreme, Mokrasch (11) reported that the color remained stable for several days if the tubes were stoppered and stored at an ice-cold temperature. In the present work (Figure 3), the maximum optical density of the anthrone glucose color was shown to occur 1.5 hr. after cooling and storage at 3° C. At 2.5 hr., the decrease in color was less than 1% and at 8.5 hr., the decrease was only 3.4%. On the basis of these data, it can be recommended that the reaction color can be read at any time from 0.5 hr. to 8.5 hr. after cooling, with the provision that standards are run concurrently.

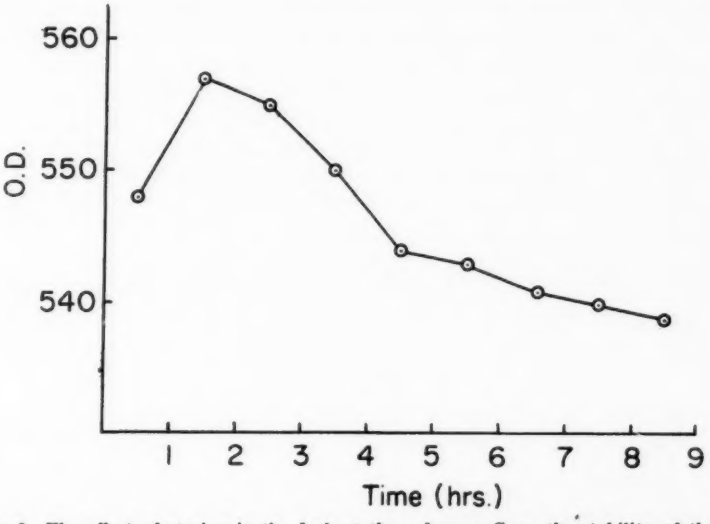

FIG. 3. The effect of storing in the dark at three degrees C. on the stability of the color produced by 1 ml. of glucose solution (100 μg/ml) and 10 ml. of 0.05% anthrone in 72% sulfuric acid. The solutions were read in a Coleman Universal Model 14 spectrophotometer, using 19- by 105-mm. matched cuvettes, at a wavelength of 620 mμ.

The keeping quality of the anthrone reagent has possibly been a limiting factor in its general acceptance. According to the literature, the following factors have been found to decrease the keeping qualities of the reagent: high concentrations of anthrone and sulfuric acid (19); exposure of the reagent to light (7) and air (19); and the use of sulfuric acid containing a high nitrate content (19). In keeping with the foregoing recommendations, we prepared reagents using low concentrations of anthrone and sulfuric acid (0.05% in 72% sulfuric acid) and stored them in glass-stoppered amber bottles at three degrees C. Figure 4 indicates that there was a slight decrease in the optical density of the anthrone-glucose color with time. The readings of the reagent blank also increased only slightly over the 30-day period. In the only other

work of this nature, Fraga (5) reported similar results; however, he used 0.17% anthrone in 84% sulfuric acid. These changes in the reagent with time preclude the use of a standard curve. It is a simple matter to include standards with each series of samples to remove the day-to-day reagent variation.

Durham (3) added known amounts of glucose to protein-free blood filtrates and recovered 98-101%. In a similar experiment, we added known amounts of glucose to whole blood, so that 1 ml. of the respective protein-free filtrates contained 0, 25, 50, and 100 μg/ml. The recovery was 96 to 104%. Our experiments were repeated four times with blood from four different animals.

There have been previous comparisons made between the use of the anthrone reagent and various copper reduction methods for the analysis of blood glucose; however, there appears to be no agreement as to whether there is a difference between the results of the two methods (3, 8, 10, 14, 16, 18). In an attempt to

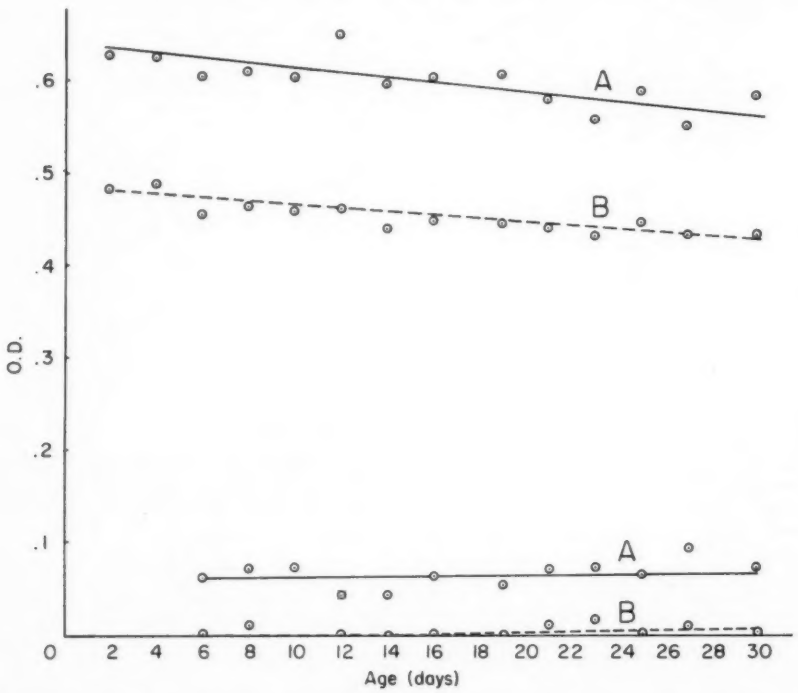

FIG. 4. The effect of storage in the dark at three degrees C. on the keeping qualities of the anthrone reagent (0.05% anthrone dissolved in 72% sulfuric acid). The upper lines refer to the optical density of solutions composed of 1 ml. of glucose solution (100 μg/ml) and 10 ml. of anthrone reagent carried through the standardized procedure. The lower lines refer to the optical density of the anthrone reagent itself compared against a distilled water blank. The solutions were read at a wavelength of 620 mμ in a Coleman Universal Model 14 spectrophotometer (Lines A) and a Bausch & Lomb Spectronic 20 spectrophotometer (Lines B), using matched 19- by 105-mm. and ½-in. cuvettes, respectively.

clarify the situation, a group of 200 comparisons was randomly drawn from a pool containing the results of the glucose analyses of 1,517 protein-free blood filtrates. Each comparison included the result from the use of the anthrone reagent, as well as the result of the copper reduction method (12, 17). It was found by statistical analysis that the difference between the results of the two techniques was highly significant (1% level). The anthrone technique yielded the higher values. A correlation coefficient of 0.94 was found between values obtained by the two methods. The inherent error in both methods was the same, as indicated by almost identical standard deviations.

As a result of our experience with the anthrone reagent in the analysis of blood protein-free filtrates for their sugar content, we have adopted and recommended the following procedure: One milliliter of protein-free filtrate is pipetted into an 18- by 150-mm. culture tube and capped with a glass marble. The tube is placed in an ice–sodium chloride slurry bath and cooled. Ten milliliters of ice-cold anthrone reagent (0.05% anthrone in 72% sulfuric acid) are run directly into the filtrate while it is still in the ice bath, and swirled briefly. The tubes containing the anthrone and filtrate are dipped in a tap-water bath for 3 min., then immersed in a covered bath of boiling water for 10 min. At the end of 10 min., the tubes are removed from the boiling-water bath and cooled in a tap-water bath for 3 min. The tubes are taken from the tap water and placed in the dark to permit full color development without photochemical decomposition of the anthrone. After 30 min., the contents of each tube are poured into matched spectrophotometer cuvettes or cells and read in a spectrophotometer at a wavelength of 620 mμ.

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PRESERVATION OF BOVINE SPERMATOZOA AT −79 AND −196° C.[1]

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SUMMARY

Studies were conducted primarily to determine the effect of storage temperatures of −79 and −196° C. on fertility of bovine spermatozoa.

A total of 145 ejaculates from 43 bulls of the Holstein, Jersey, Guernsey, Angus, Ayrshire, and Brown Swiss breeds were frozen in an egg yolk–citrate extender. The 60- to 90-day per cent nonreturn (NR) rate from 3,214 first-service inseminations with liquid nitrogen (LN) stored semen was 70.6, compared to 3,091 first services with a NR rate of 67.8% from DI storage. The weighted mean difference in per cent NR of 2.9 was statistically significant ($P < 0.02$).

Breeding efficiencies were obtained on second-service inseminations from the two treatments. The number of services and 60- to 90-day per cent NR was 1,132, 68.1 versus 1,143, 66.9 for LN and dry ice–alcohol (DI) stored semen, respectively. The weighted mean difference of 1.1% NR in favor of LN was not significant ($P = 0.05$).

In a second field trial, semen from nine Holstein bulls was frozen in LN, to −80° C., and then divided equally for storage at −79 and −196° C. A total of 299 first-service cows were bred with semen stored in LN for a 60- to 90-day NR of 73.2%, as compared to a NR rate of 67.6% on 330 first services to DI stored semen. There was a weighted mean difference of 5.3% in favor of LN. The difference was not statistically significant at $P = 0.05$.

Combined data from this station included a total of 9,742 first-service cows, 4,951 with semen stored in LN versus 4,791 with semen stored in DI. The 60- to 90-day per cent NR was 71.1, compared to 68.4 for LN and DI, respectively. There was a significant difference ($P < 0.01$), indicating that LN, under the conditions of these studies, is a superior refrigerant for storage of bovine spermatozoa.

Thirteen measurements were collected on each of 132 ejaculates. These characteristics were correlated with fertility of semen stored in DI and LN. All correlations were too low to be useful in predicting the fertility of frozen semen.

The use of liquid nitrogen (LN) as a refrigerant for storage of bovine spermatozoa has received considerable attention in recent years (2, 7-9, 11-14). Sufficient evidence has accumulated to show definitely that per cent progressive motility is maintained at a higher level in semen samples stored in LN at −196° C. than split-samples stored in dry ice–alcohol (DI) at approximately −79° C. (2, 7, 8, 11, 13). While statistically significant differences ($P = 0.05$) in fertility between the two storage temperatures have not been obtained, data support the view that a higher nonreturn rate can be expected from bovine semen stored in LN. For example: Larson and Graham (7) reported a 2.6% difference in 75-day nonreturns in favor of the colder temperature. Macpherson

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(9) found a difference in 60- to 90-day nonreturns of approximately 2% in favor of LN, and Pickett *et al.* (12) showed LN to have an advantage of 1.61% in nonreturns over DI storage. The absence of significant differences was possibly because of too few cows and bulls being used in any one experiment. It was of interest that in these studies the nonreturn rates for LN stored semen range from 71.9 to 71.43%, whereas the DI nonreturns ranged from 70.14 to 69.3%.

Because of the above-mentioned encouraging results with LN as a refrigerant for bovine semen, the present study was undertaken primarily to extend these findings, especially with respect to insuring adequate numbers, to ascertain possible fertility differences that might exist between −79 and −196° C. storage of bovine spermatozoa.

In all controlled LN vs. DI field studies reported to date (7, 9, 12), the semen has been frozen in DI and the portion for LN storage transferred to LN immediately after freezing. Consequently, a field trial was conducted to explore the effects of freezing semen in LN for LN and DI storage.

Previous communications (5, 15) have indicated that only limited studies have been conducted to aid in selecting superior frozen semen samples prior to shipment to the technicians. Therefore, during the large-scale field trial, certain characteristics were observed on each ejaculate and correlated with fertility of semen stored in LN and DI.

EXPERIMENTAL PROCEDURE

A total of 145 ejaculates of semen was obtained during February-March, 1960, from 43 bulls of the Holstein, Jersey, Guernsey, Angus, Ayrshire, and Brown Swiss breeds routinely used at the New England Selective Breeding Association, Woodbridge, Connecticut. In some cases, several ejaculates from a particular bull, collected on any one day, were pooled and considered for purposes of this study as an ejaculate. No selection of bulls was practiced, since no technicians received liquid semen during the course of the experiment. All collections for these studies were in addition to those needed for routine shipment of liquid semen. Immediately after collection, the semen was estimated microscopically for per cent progressively motile cells, evaluated photometrically for sperm concentration, and extended to contain, after glycerolation, 30 million progressively motile cells per milliliter of extender. The final concentrations of egg yolk, sodium citrate, glycerol, and antibiotics were as previously outlined (11).

The procedures consisted of extending the semen to one-half the final volume at 32° C., placing in beakers of water maintained at 32° C., and allowing to cool for approximately 3.5 hr. to 5° C. The glycerol-containing fraction of the extender was then added in four equal portions at 15-min. intervals. After the semen had been glycerolated, the flasks were adequately covered, packed in styrene semen shippers, maintained at 5° C., and transported approximately 70 mi. to the University for further processing. Toward the end of the 15-hr. equilibration period, the semen was hermetically sealed in 1.2-ml. glass ampules

and frozen, in a dry ice–alcohol bath, on racks designed for liquid nitrogen storage (19). The freezing rate used in this study was approximately 1° C. per minute from 5 to −15° C. and 4° C. per minute from −15 to −79° C. Immediately after freezing, the ampules were divided equally and one-half was transferred to liquid nitrogen refrigerators (Linde Company Model LNR-25-B) maintained at −196° C.; the remaining one-half was transferred to units held at −79° C. with DI.

The frozen semen was distributed by University personnel to 17 technicians in Connecticut, Massachusetts, and Vermont. The technicians, previously divided into two groups, were provided with frozen semen on a full-time basis during April-May, 1960. Regardless of refrigerant, all semen was dispensed to technicians in LNR-25-B field storage units. Ampules from each ejaculate were arranged so that each technician used semen from one storage temperature and the following month switched to split-ejaculates of semen stored at the other temperature. Technicians were instructed to thaw the ampules in ice water and use as quickly as possible after thawing, but under no circumstances to use the semen if it had been thawed longer than 3 hr. Ampules were coded, as well as the racks holding them. Technicians were instructed to record both codes on the breeding receipt sent to the organization office. Under these conditions, if either code did not agree with the sire's name, the service was discarded. Fertility comparisons were based on 60- to 90-day per cent nonreturns.

A limited field trial was conducted to investigate the effects of freezing semen in LN prior to storage in LN and DI. The experimental design was similar to that outlined above, with the following exceptions: (a) Semen samples from nine Holstein bulls were frozen in a liquid nitrogen semen freezer to −80° C. (Linde Company Model BF-1), and divided for storage at −79 and −196° C.; (b) the frozen semen was delivered to the University for distribution; (c) most of the semen was collected in June, 1960, and the field trial was conducted with only six technicians in July and August, 1960.

During the larger of the two field studies described above, data were collected on sperm concentration, volume, motility (initial, prefreeze, immediate post-freeze, and after 3-wk. storage), and number of sperm surviving freezing and storage. Each of these criteria were used to calculate a weighted correlation with fertility by the method of Quenouille (17), which takes into account the different number of services per ejaculate.

RESULTS AND DISCUSSION

The 60- to 90-day per cent nonreturns, by breed and by storage method, are presented in Table 1. A test of significance of the effect of storage temperatures on fertility was performed, using the weighted "t" test (18) for each breed and for the average across all breeds. The latter resulted in a mean difference between storage methods of 2.9% in favor of LN ($P < 0.02$). These results agree very favorably with those previously reported (7, 9, 12), except that in this case a statistically significant difference was obtained which was probably due to the greater numbers of cows and bulls used in the present study.

TABLE 1

The 60-90 day per cent nonreturn rate of semen stored in liquid nitrogen and dry ice–alcohol

Breed	No. Bulls	No. Ejaculates	Liquid nitrogen No. of 1st services	Liquid nitrogen % NR 60-90	Dry ice–alcohol No. of 1st services	Dry ice–alcohol % NR 60-90	Weighted difference	S.E.[a]
Holstein	13	52	1,778	70.8	1,641	67.2	+ 3.5*	1.3
Jersey	10	35	660	73.2	645	72.3	+ 1.1	2.4
Guernsey	9	28	377	65.3	347	60.2	+ 4.7	4.2
Angus	4	14	247	71.7	299	74.3	− 2.5	1.6
Ayrshire	5	12	127	70.9	124	61.3	+10.1	8.9
Brown Swiss	2	4	25	64.0	35	57.1	+ 3.4	13.0
Total	43	145	3,214	70.6	3,091	67.8	+ 2.9*	1.2

* Significant at $P < 0.02$.
[a] Standard error of the weighted mean difference.

Also of possible interest was that semen from 32 of 43, or 74.4%, of the bulls exhibited a higher conception rate in LN than when stored in DI. Upon examination of these data, by technician for each treatment, it was noted that the 60- to 90-day per cent nonreturns ranged from 77.8 to 61.1% for LN and from 79.0 to 57.7% for DI storage. While this difference suggested a more uniform nonreturn rate among technicians using LN stored semen, the test for comparing weighted variances (20) indicated the LN nonreturn rate was not significantly more uniform.

Data obtained on second services are presented in Table 2. The weighted mean difference of 1.1% in favor of LN stored semen was not statistically significant ($P = 0.05$). The relatively limited number of services could account for the lack of consistency among the weighted mean differences.

Data are presented in Table 3 indicating the effect of freezing of semen in LN and subsequent storage in either LN or DI. There was a weighted mean difference in 60- to 90-day nonreturn rate of 5.3% in favor of the colder temperature ($P < 0.20$). This level of significance is not sufficient to make a firm

TABLE 2

Per cent 60-90 day nonreturn rates on second-service inseminations to semen stored at −79 and −196° C.

Breed	No. bulls	Liquid nitrogen No. of 2nd services	Liquid nitrogen % NR 60-90	Dry ice–alcohol No. of 2nd services	Dry ice–alcohol % NR 60-90	Weighted difference	S.E.[a]
Holstein	13	618	68.3	586	65.9	+1.9	1.9
Jersey	10	221	71.0	222	74.3	−3.6	6.1
Guernsey	9	131	66.4	147	58.5	+7.4	5.6
Angus	4	103	67.0	109	69.7	−0.5	9.6
Ayrshire	5	43	60.5	61	63.9	−3.4	13.9
Brown Swiss	2	16	68.8	18	72.2	−5.9	4.2
Total	43	1,132	68.1	1,143	66.9	+1.1	2.1

[a] Standard error of the weighted mean difference.

TABLE 3

Comparison between the 60-90 day nonreturn rates of bull semen frozen in liquid nitrogen and stored at −79 and −196° C.

	Liquid nitrogen		Dry ice–alcohol	
Bull	No. of 1st services	% NR 60-90	No. of 1st services	% NR 60-90
125	28	75.0	31	77.4
141	14	92.9	14	78.6
144	10	40.0	18	72.2
151	38	76.3	32	68.8
152	16	62.5	20	55.0
157	82	74.4	78	64.1
139	46	69.6	61	70.5
169	38	71.1	47	63.8
173	27	81.5	29	65.5
Breed total	299	73.2[a]	330	67.6[a]

[a] Weighted mean difference between 73.2 and 67.6 = 5.3, with a standard error of 3.3; difference not significant at $P = 0.05$ ($P < 0.20$).

commitment as to the superiority of the colder storage temperature, probably because of the limited numbers involved. In any event, these data indicate that as good, or better, conception rate can be obtained with semen frozen in LN for LN storage than would be expected from DI frozen semen subsequently stored in LN. However, in the studies reported herein, adequate controls were not included to compare LN freezing to DI freezing of semen to be stored in LN.

It has been suggested (6, 16) that bull semen to be frozen and stored in LN may need to be treated differently than semen for DI storage. The most obvious difference resulting from the two methods of freezing is the ice coat formed around the ampule during thawing. When ampules of semen frozen in DI and stored in LN are thawed in ice water (+5 to 0° C.), the ice that forms around the ampule melts quickly, due to contamination of the ice with alcohol. However, when semen is frozen in LN and stored in LN, the ice coat formed in the ice water is hard and does not melt away completely until the ampule has thawed. It is possible that this harder ice coat provides a more uniform thawing temperature for the semen.

In an attempt to provide as much information as possible on LN versus DI storage of frozen semen, all available data from this station comparing LN to DI have been combined and are presented in Table 4. To date, three field studies have been conducted at this station, employing the same experimental design, i.e., split-ejaculates were divided for storage in DI and LN, in LNR-25-B LN refrigerators, and each technician had an equal opportunity to use split-ejaculates on first-service cows, stored at both temperatures.

Experiments 2 and 3, shown in Table 4, are described herein, whereas Experiment 1 has been reported elsewhere (12). The 2.7% nonreturn weighted mean difference in favor of LN was significant at the 1% level of probability. These results show that, under the conditions of these studies, LN is a superior refrigerant for storage of bovine spermatozoa.

TABLE 4

Combined data from three studies conducted at this station investigating dry ice–alcohol and liquid nitrogen storage of frozen semen

Expt. No.	No. Breeds	No. Bulls	Liquid nitrogen: No. of 1st services	Liquid nitrogen: % NR 60-90	Dry ice–alcohol: No. of 1st services	Dry ice–alcohol: % NR 60-90	Weighted difference	S.E.[a]
1	4	15	1,438	71.6	1,370	70.1	1.6	1.5
2	6	43	3,214	70.6	3,091	67.8	2.9*	1.2
3	1	9	299	73.2	330	67.6	5.3	3.3
Total		67	4,951	71.1	4,791	68.4	2.7**	0.9

* $P < 0.02$.
** $P < 0.01$.
[a] Standard error of the weighted mean difference.

The means, standard deviations, and coefficients of correlation between certain semen characteristics and fertility are shown in Table 5. Although some significant correlations were obtained, all coefficients were too small to be of value in predicting the fertility of frozen semen. Higher coefficients of correlation would probably have been obtained if fewer than 30 million spermatozoa

TABLE 5

Characteristics of 132 semen samples from 43 bulls and their correlation with fertility

Characteristic	Mean	S.D.[b]	Correlation: LN	Correlation: DI
Fresh semen				
Sperm concentration/ml (10^6)	1,292.70	496.59	0.06	−0.02
Volume *(ml.)*	7.36	3.28	0.02	0.06
Initial motility[a] *(%)*	62.61	7.62	−0.05	−0.05
Extended semen				
Motility—Prefreeze *(%)*	61.58	8.55	0.11	0.00
Motility—Post-freeze *(%)*	43.99	9.63	0.19*	0.09
Per cent survival	71.69	13.62	0.14	0.10
No. sperm surviving (10^6/ml)	21.22	4.53	0.22*	0.12
Storage (3 wk.)				
DI				
Motility *(%)*	33.11	7.92		0.04
Per cent survival	54.18	12.49		0.05
No. sperm surviving (10^6/ml)	16.00	3.87		0.07
LN				
Motility *(%)*	42.46	8.98	0.08	
Per cent survival	69.55	14.60	0.01	
No. sperm surviving (10^6/ml)	20.53	4.34	0.12	
Fertility data				
Liquid nitrogen				
No. services/ejac	23.85	18.88		
Per cent nonreturn (60-90)	70.71	9.52		
Dry ice–alcohol				
No. services/ejac	23.35	17.64		
Per cent nonreturn (60-90)	67.91	10.89		

* Significant at $P < 0.05$.
[a] All motility values are expressed in per cent progressively motile spermatozoa.
[b] Standard deviation.

per milliliter of extender had been used (4), or if a storage time exceeding 2 to 4 mo. had been employed (1, 3, 10). Correlations were probably reduced due to the large number of cells per milliliter of extender and the semen being used soon after freezing, especially since a rather large difference between number of sperm surviving 3-wk. storage in LN as compared to DI was observed (Table 5).

Results from these studies indicate that an acceptable conception rate can be obtained with frozen semen provided ejaculates possessing a per cent progressive motility of less than 50 are discarded upon collection, semen is extended to contain 30 million progressively motile cells per milliliter, and ampules are discarded after freezing if more than two-thirds of the cells are immobilized during the freezing and thawing process.

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TECHNICAL NOTES

RELATIONSHIP BETWEEN AREAS UNDER ULTRACENTRIFUGAL SEDIMENTATION PATTERNS AND CONCENTRATION FOR MILK PROTEINS

Interpretation of ultracentrifugal results often requires estimation of concentrations from areas defined by sedimentation patterns. This is especially true in studies of unknown mixtures, such as may be produced by heating milk, and of known mixtures in which interactions may occur. Although proteins are basically similar in structure, and presumably similar in refractive index, there is need of assurance as to the limits within which constant proportionality between area and concentration may be assumed. Accordingly, many sedimentation patterns on known systems were accumulated and remeasured. Essential results are shown in Figures 1 and 3. The conclusion is that for all small molecules, and even for moderately large ones ($S = 100 \times 10^{-13}$), the concentration/area factor has a single definite value within narrow limits; but that above $S = 100 \times 10^{-13}$, approximately, the factor may be expected to increase with particle size. The breakdown in constant proportionality occurs at particle diameters of the order of 500Å, roughly coincident with onset of light scattering.

All patterns analyzed were obtained with the Spinco Model E ultracentrifuge,[1] most using the standard 12-mm. cell, but some using the synthetic boundary cell, and one (for ovalbumin) using a 1.5-mm. cell.

Only patterns for which a base line was clearly defined or was separately determined were included. Tracings on heavy 1-in.-1/10-

[1] Mention of trade names is for the purpose of identification only and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

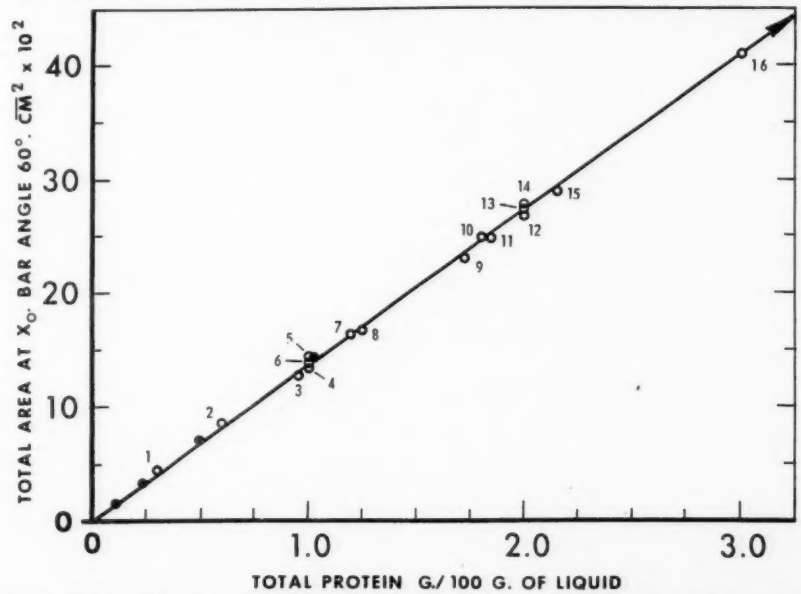

FIG. 1. Relationship of areas under Schlieren patterns to concentration for various proteins. The four solid circles are for bovine serum albumin in Veronal, pH 8.6. The numbered open circles represent: (1) snail blood, *Otala lactea*, $S = 89 \times 10^{-13}$; (2) α-casein, pH 11.2; (3) centrifuge milk serum, dialyzed against Veronal, pH 8.5, three components resolved; (4) α-casein complex fraction, washed in water and dialyzed against Veronal, pH 8.3, four components resolved; (5) centrifuge milk serum, dialyzed against Veronal, pH 8.5; (6) β-lactoglobulin, pH 6.8, two components unresolved; (7) α-casein, pH 12.2; (8) ovalbumin in 0.05 *M* NaCl; (9) small size fraction of the casein complex, washed in water, dialyzed against Veronal, pH 8.4, three components resolved; (10) medium-sized fraction of the casein complex, washed in water, dialyzed against Veronal, three components resolved; (11) large-sized fraction of the casein complex, washed in water, dialyzed against Veronal, three components resolved; (12) α-casein in Veronal; (13) bovine serum albumin plus γ-globulin, 1% each, pH 6.6; (14) large-sized fraction of the casein complex, washed, dialyzed against Veronal, three components resolved; (15) α-casein complex fraction treated with sodium tetrametaphosphate at pH 8.6; (16) α-lactalbumin plus β-lactoglobulin, 1.5% each, pH 6.9, two components resolved. The line through these various points is drawn with an arrow because the ovalbumin area (8) is actually calculated from the area for a 10% solution in a 1.5-mm. cell.

inch cross-ruled paper were prepared by use of an enlarger at a magnification of 6.39 cm. per centimeter in most cases. Either the left-hand reference line or the meniscus was set on a heavy (1-in.) vertical line, and the lower margin of the base on a heavy horizontal line. The lower margin of the complete pattern was then traced in red; next the paper was moved to set the upper margin of the base on the red tracing and the upper margin of the complete pattern then traced in blue. Finally, an average, black outline was drawn between the red and blue tracings. For this a fine ball-point pen is convenient, since it leaves a neat track for the planimeter needle.

Each planimeter tracing consisted of six complete circuits of the pattern; the instrument being read after the first and sixth circuits. The difference between these readings divided by five was taken as the area. Such measurements were always made at least in duplicate, usually several times. The average mean deviation of such average area measurements is 0.165×10^{-2} $\overline{cm}^2$ on the scale of Figures 1, 2, and 3, making the average error for small proteins 1% at a concentration of 1.2%, for example. The first circuit in a set of six is discarded, because it is found to be often in error, due to backlash and for other reasons.

In the various centrifugations bar angles as low as 30° and as high as 70° were used. All areas were corrected to a bar angle of 60° by multiplying by the factor tan (90°-60)/tan (90°-θ). No deviations in areas attributable to changes in bar angle were observed, even at concentrations as low as 0.1%.

All areas given in Figure 1 are at zero equivalent centrifuging times, i.e., at zero fall distance. Extrapolations were made by plotting the measured areas from successive frames of a set against fall distance ($X_{peak} - X_{meniscus}$), and by plotting the measured areas against $X_p^2/X_{men.}^2$. Both extrapolations tend to give the same areas at zero equivalent centrifuging time.

The solid lines of Figure 2 show three typical plots of areas as actually measured: the dashed lines are the areas multiplied by X_p^2/X_m^2, the theoretical correction for radial dilution. In only one case, for ovalbumin, is the theoretical correction adequate. Not only may the theoretically corrected plots slope either up or down

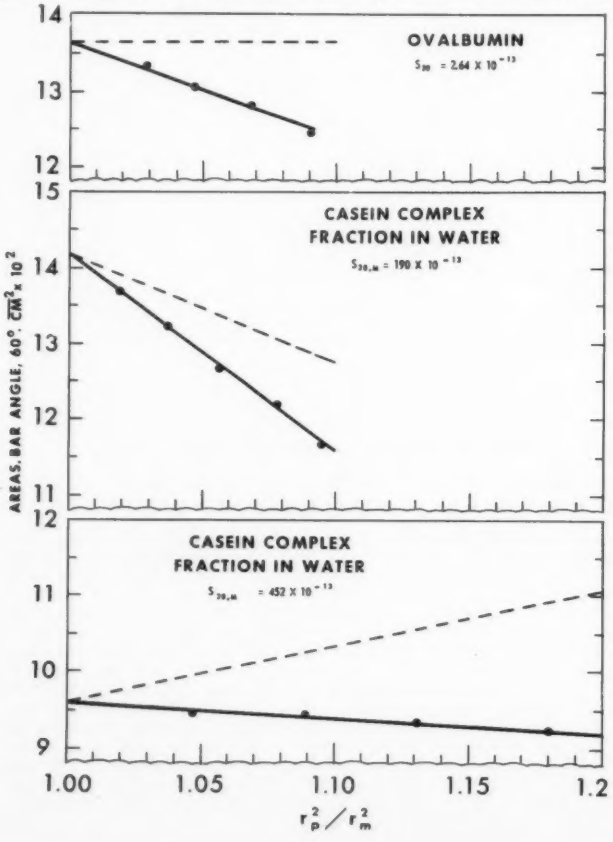

FIG. 2. Typical plots of measured areas against X_p^2/X_m^2, together with the areas corrected for theoretical radial dilution by this factor, showing the magnitude of errors possible by using corrected areas for single patterns.

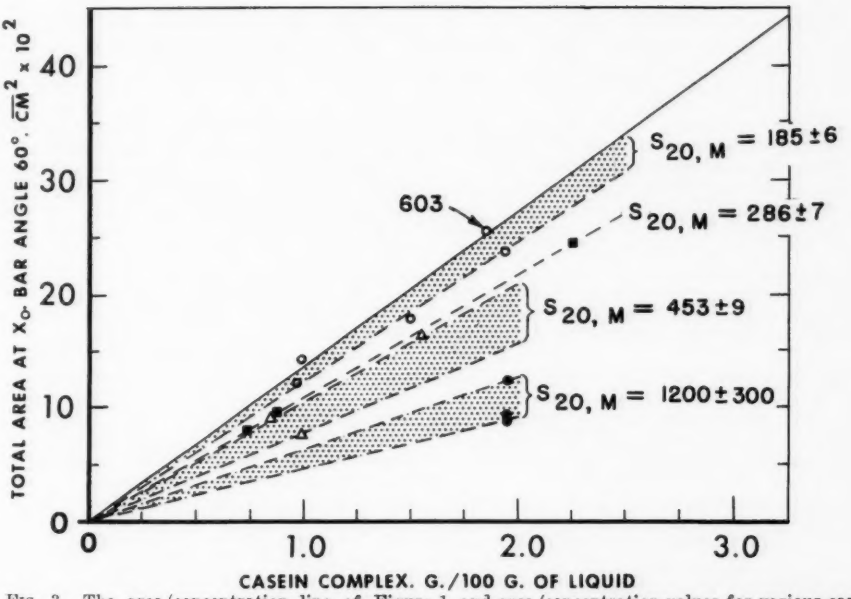

FIG. 3. The area/concentration line of Figure 1, and area/concentration values for various casein complex fractions prepared by centrifuging. These fractions were all washed with water, dispersed in water, and the dispersions all found to be at pH 7.5, approximately. The fractions represent narrow particle size ranges but are still polydisperse. The sedimentation constants given are the dominant values corresponding to the peaks of the Schlieren patterns. These sedimentation constants have been corrected to the viscosity and density of average milk serum, and to the average voluminosity of the casein colloid in milk. The values observed, in water, are 2 to 50% higher than the corrected values given.

but they are sometimes curved. These deviations may be attributed to polydispersity, asymmetry, gelation, etc. Detailed discussion will be presented elsewhere. For the present purpose, it is sufficient to note that correction of single patterns by the theoretical radial dilution factor may lead to large errors. Some sort of extrapolation to zero equivalent centrifuging time is essential.

Multiple peaks when resolvable should be plotted separately and the intercepts at $t = 0$ added together as a check on the total area. In cases where a pattern is clearly resolved, and symmetrical, but one leg is partly hidden, as occurs near the meniscus and again near the base of the cell, the truncated areas thus defined will be low. If these outlines be completed, however, by drawing in the mirror image of the leg which is definite or can be resolved, then the area of the new pattern will fall in line with the others.

Figure 3 is of especial interest with respect to the casein complex. It is noted first that the sedimentation constants 185, 286, and 453 correspond to particle volumes in the ratio 1:2:4, approximately. This follows from Stokes' law, $r = \sqrt{\frac{9}{2} \frac{\eta s}{(\rho_p - \rho_m)}}$. The departure of the indicated concentrations from the actual concentrations are roughly in the same ratios. This would be expected, since at a given concentration light scattering is theoretically proportional to particle volume. It will be noted also that for each average s value the deviations in areas are much greater than the deviations in s. For each of these fractions the voluminosity, the ratio of the total particle volume to the volume of contained protein, was calculated from viscosity measurements.[2] In general, for a given s value, the preparations having high voluminosities deviate least from the correct area/concentration line. In the one preparation designated by Experiment No. 603, the voluminosity found was 13, as compared with an average value of 4.3 for the colloid in normal milk. In this case, therefore, a loose structure is indicated, probably sufficiently transparent to light as to have the same effect in light bending as if it were a collection of small protein molecules.

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AMINO ACID REQUIREMENTS OF LEUCONOSTOC CITROVORUM AND LEUCONOSTOC DEXTRANICUM ISOLATED FROM DAIRY STARTERS AND CULTURED MILK PRODUCTS [1]

Although *Leuconostoc citrovorum* and *Leuconostoc dextranicum* are important species of dairy starters, because of their role in the production of biacetyl, little information is available on the nutritional requirements of these species. Limited studies (1-4), involving only a few strains, have been made of the amino acid requirements of these species. One of the strains studied, *L. citrovorum* ATCC 8081, later was shown to be a typical strain of *Pediococcus cerevisiae* rather than a strain of *L. citrovorum* (2).

This study was undertaken with the hope that additional information concerning the amino acid requirement would be useful in developing a plating medium by which *L. citrovorum* and *L. dextranicum* could be differentiated in cultured dairy products.

The data presented in Table 1 represent a study of 18 strains of *L. citrovorum* and 15 strains of *L. dextranicum* isolated from dairy starters and cultured milk products.

The basal synthetic medium was that used by Whiteside-Carlson and Rosano (3), with the addition of cystine and glutamine.

The cultures were grown for 24 hr. on standard plate count agar slants, the growth removed to centrifuge tubes, and washed and suspended three times, using a sterile physiological saline solution. A loop of the suspended culture was transferred to a 2-ml. portion of the test medium and incubated for a period of ten days at 25° C. Four observations were made with each culture. The development of acid as measured by a quinhydrone electrode was used as the criterion of growth.

The amino acid requirements were very similar for the strains of the two species. All strains of *L. citrovorum* required arginine, histidine, isoleucine, leucine, lysine, and valine. In addition, all but one strain required phenylalanine and tryptophan. All strains of *L. dextranicum* required isoleucine, leucine, valine, phenylalanine, and tryptophan. Arginine, histidine and lysine each was required by all but one strain and in the case of each a different strain was involved.

On the basis of the amino acid requirements, the 18 strains of *L. citrovorum* fell into 12 groups and the 15 strains of *L. dextranicum* into nine. The minimum and maximum numbers of amino acids required for the strains of both species were seven and 15, respectively. Dunn *et al.* (1), in a study of three strains of *L. citrovorum*, found the number of required amino acids to be two, two, and seven, respectively. For three strains of *L. dextranicum* they found these to be three, seven, and seven.

[1] This investigation was supported in part by funds provided for Biological and Medical Research by the State of Washington, Initiative Measure No. 171.

TABLE 1

Amino acid requirements of strains of *Leuconostoc citrovorum* and *Leuconostoc dextranicum*

Amino Acid	Strain of *L. citrovorum*												Strain of *L. dextranicum*								
	1-6	7-8	9	10	11	12	13	14	15	16	17	18	1-6	7-8	9	10	11	12	13	14	15
Arginine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Isoleucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Valine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenylalanine	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tryptophan	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tyrosine	−	+	−	−	−	+	+	−	+	−	+	−	−	+	−	−	−	−	−	+	+
Cysteine	−	−	−	+	+	+	−	−	+	−	+	+	−	+	+	−	−	−	−	+	−
Glutamine	−	−	−	−	+	−	−	−	+	+	+	−	−	−	+	−	−	−	+	−	−
Glutamic Acid	−	−	−	−	−	−	+	+	−	+	+	−	−	−	+	−	−	+	+	−	+
Proline	−	−	−	−	−	+	+	−	+	−	+	−	−	−	+	−	−	−	−	+	+
Alanine	−	−	−	−	+	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−
Serine	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Threonine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Asparagine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Aspartic Acid	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glycine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Methionine	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+
Cystine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−

+ Required for growth; − Not required for growth.

Threonine, asparagine, aspartic acid, glycine, and cystine were not required by any strain of *L. citrovorum*. Methionine was required by only one and alanine and serine by two. Serine, threonine, aspartic acid, and glycine were not required by any strain of *L. dextranicum*. Alanine, asparagine, and cystine each was required by one strain. Glutamine and methionine each was required by two strains.

The amino acid requirements do not appear to be of value as a criterion for differentiating these two species of bacteria.

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STABILIZATION OF CALCIUM-SENSITIVE (α_s) CASEIN BY KAPPA-CASEIN: EFFECT OF CHYMOTRYPSIN AND HEAT ON KAPPA-CASEIN

It has been found helpful in studies of the caseins to characterize the calcium-sensitive casein by its quantitative precipitation with calcium chloride (6), and the kappa-casein by its ability to quantitatively stabilize the calcium-sensitive casein so that it does not precipitate with calcium chloride (5). The latter test, which has not been reported in detail, is performed with calcium-sensitive (α_s) casein (0.3%), at pH 6.7, with varying amounts of kappa-casein, with a 0.020 *M* concentration of calcium chloride. The test is set up in 15-ml. centrifuge tubes; total volume of the test mixture is 10.0 ml. A 2% solution of the α_s-casein at pH 7.8 is used; subsequent addition of calcium chloride brings the pH of the mixture to the desired pH of 6.7. The solution of α_s-casein (30 to 34 mg.) is added to the tubes, followed by the required volumes of water, and amounts of kappa-casein such that the kappa/α_s ratios vary from about 0.03 to 0.12. Finally, 2.0 ml. of 0.1 *M* calcium chloride is added and the mixture stirred with a spatula. The test mixtures are kept at 30° C. for 15 min., then centrifuged at about 3,000 × G for 5 min. Samples of the supernatant solutions are withdrawn, appropriate dilutions made, and 1 drop of 0.5 *M* NaOH added to clarify the dilutions. The casein in solution is determined from the light absorption at 280 mμ. A factor of 1.0 is used for convenience for converting light absorption (optical density) to milligrams of protein per milliliter. The amount of kappa-casein used in the test is deducted from the casein in solution and the results expressed as per cent of α_s-casein in solution. Solutions of α_s-casein for the test are stored at 7° C. and are not kept more than three days, since the soluble portion at zero kappa concentration tends to increase. A stabilization test with an aged α_s-casein solution parallels that obtained with a fresh solution, but is at a higher level. A typical curve for kappa-casein by this test procedure is shown by the curve No. 1 (O———O) in Figure 1-A, where the per cent α_s-casein soluble is plotted against the ratio of kappa/α_s in the test mixtures. Choice of a method for preparing kappa-casein of maximum stabilizing power has been guided by the use of this stabilization test.

This test has also been useful in investigating the influence of various treatments on kappa-casein. One of the treatments investigated was the influence of heat (90° C. for 15 min.). On investigating various preparations of kappa-casein it was found that some were heat-stable, that is, after heating they retained their ability to stabilize the calcium-sensitive casein, whereas others were heat-labile. The latter were adequate stabilizers before heating, but after heating had lost much or all of this property.

The explanation for this difference in the effect of heat on kappa-casein is not apparent yet. Chemical manipulation during the preparation of the kappa-casein might produce the heat-labile form, or enzymes in milk might produce the heat-labile form. The protease in milk is a possibility, since this enzyme is precipitated almost quantitatively with the casein in the preparation of acid-precipitated casein (4). This protease is of the trypsin-chymotrypsin type, most active at pH 8.5. The enzymes trypsin and chymotrypsin are considerably less specific in their action on casein than are pepsin and rennin (2). Chymotrypsin was available, so the effect of this enzyme on the stabilizing activity of kappa-casein has been investigated. The effect of heat on the partially enzyme-altered kappa-casein also has been

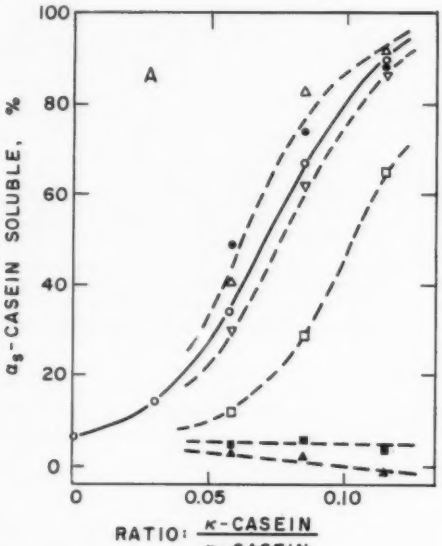

FIG. 1. Stabilization of calcium-sensitive (α_s) casein by kappa-casein. Influence of heat and chymotrypsin treatment on kappa-casein. Details of the test are described in the text. Results with kappa-casein prepared by the method of McKenzie and Wake (3) and subsequently ultracentrifuged to remove the heavy fraction (results by Dr. M. P. Thompson have shown that the heavy fraction has very little stabilizing ability). Stabilization tests with the kappa-casein subjected to various treatments are designated by the following curves: (1) ○———○ Kappa-casein as is (1.0% stock solution at pH 7.0 used). (2) △------△ Kappa-casein heated at 90° C. for 15 min. (7). (3) ●------● Kappa-casein plus chymotrypsin (10.0 ml. 1.0% kappa-casein, pH 7.0 and 0.3 ml. (3.5 γ) of chymotrypsin, at 25-27° C.), tested for stabilization immediately. Same as curve drawn for 2. (4) △------△ Same as (3), but tested after chymotrypsin had acted for 60 min. (5) ■------■ Same as (4), but heated at 90° C. for 15 min. before tested. (6) □------□ Same as (3), but tested after chymotrypsin had acted for 120 min. (7) ▲------▲ Same as (6), but heated at 90° C. for 15 min. before tested.

studied. The results with two preparations of kappa-casein are given in Figures 1 and 2.

The experiments described utilize amounts of chymotrypsin (ratio of kappa-casein to chymotrypsin 28,500 to 1) that have only a limited action on casein. Calcium caseinate is clotted by chymotrypsin (2) and the present studies have shown that kappa-casein can be clotted with chymotrypsin (500:1 at pH 6.8) with or without calcium chloride present. The kappa-casein, inactivated by successive action of chymotrypsin and heat as described in the present experiments, is clotted by calcium chloride at concentrations of 0.005 *M*, but this clot dissolves at concentrations of 0.025 to 0.050 *M* (this has varied with different preparations) calcium chloride. This clot can also be dissolved with sodium chloride. Furthermore, this inactive kappa-casein, with a concentration of calcium chloride in which it is soluble, will clot when acted on by rennin.

Figure 1 shows the stabilization by a preparation of kappa-casein that is not affected by heat alone (△----△) (compare with ○———○; the apparent increase in stabilization by heating is probably not significant). When acted on by chymotrypsin for 60 min. there is a slight decrease in stabilization △----△)), and after 120 min. a considerable decrease (□----□). Both of these solutions lose their stabilization ability when heated at 90° C. (the negative stabilization slope indicates that some of the kappa-casein is precipitated with the calcium-sensitive casein).

In Figure 2 a preparation of kappa-casein is illustrated (○———○) that has some heat-lability without chymotrypsin treatment (△———△). Treatment with chymotrypsin for increasing periods (0, 15, 30, and 60 min.),

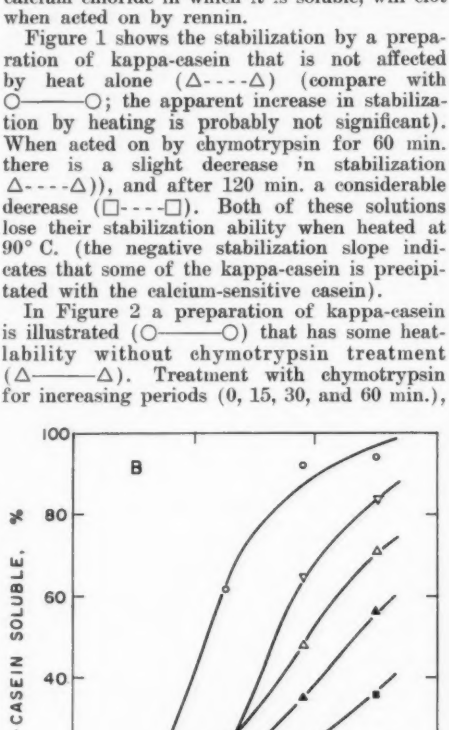

FIG. 2. Results with kappa-casein prepared by the method of McKenzie and Wake (3). This casein had not been treated to remove lipides and the solution was slightly turbid. (1) ○———○ Kappa-casein as is. (2) △———△ Kappa-casein heated at 90° C. for 15 min. (3) ▽———▽ Kappa-casein plus chymotrypsin (same conditions as for A-3). Stabilization test after chymotrypsin had acted for 60 min., no heat. (4) ▲———▲ Kappa-casein plus chymotrypsin. Immediately after addition of chymotrypsin, mixture was heated at 90° C. for 15 min. (5) ■———■ Similar to (4), but not heated until chymotrypsin had acted for 15 min. (6) □———□ Similar to (4), but not heated until chymotrypsin had acted for 30 min. (7) ▼———▼ Similar to (4), but not heated until chymotrypsin had acted for 60 min.

followed by heat, leads to increasing inactivation of the kappa-casein. At 60 min., stabilization ability is completely lost (▼———▼), whereas treatment by chymotrypsin alone has caused only partial loss of stabilization ability △———△)).

The relation of these results to the properties of kappa-casein in milk, and the properties of kappa-casein in the isolated form, can only be guessed. It does suggest, however, as a subject for further study, that the naturally occurring protease in milk might be the cause of the variable heat-lability of various preparations of kappa-casein. The protease in milk may be of the chymotrypsin type, for it has been found that free tyrosine was released by its action (1). Furthermore, the protease in milk should be studied to see if it might be responsible for some of the adverse changes that occur in the storage of high-temperature, short-time sterilized concentrated milks.

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OBSERVATIONS ON THE INFUSION OF PENICILLIN IN THE MAMMARY GLAND OF THE GOAT

The problem of antibiotic adulteration in milk has been studied in considerable detail in the dairy cow. Existing data on the cow have been the standard for producers of goat milk, despite the known and unknown differences between the two genera (5).

An udder infusion study was conducted in the University of Illinois dairy herd with 20 French Alpine dairy goats to determine: (1) The persistence of penicillin following intramammary infusion, 2) the effect of standard oil or water-base materials, 3) if differences exist between early and late lactation, and 4) if there is a transfer of penicillin from the treated to the untreated half of the udder.

The does represented various ages (1 yr. 10 mo. to 6 yr. 10 mo.), stages of lactation (17 days to 259 days post-partum), and production levels (1.6 to 9.6 lb. per day). Each doe was checked and found to be producing normal milk and was negative for antibacterial substances in the milk as shown by the disc assay test (3).

Immediately following the milking prior to the testing period, ten does were infused in the right half of their mammary gland with 100,000 units procaine penicillin G in aqueous solution. One week later the same does were infused by the same veterinarian with 100,000 units procaine penicillin G in oil suspension in the same half. All does were milked by hand twice a day to obtain a complete sample per quarter. One milker collected milk from the infused half and another milked the noninfused half as a precaution against cross-contamination of the two halves. After the milking of each half, all equipment was thoroughly rinsed in a large volume of water. The experiments were continued until the milk was antibiotic-free.

All milk samples were tested using the approved disc assay method and 0.5-in. discs for the detection of penicillin in milk given in Standard Methods for the Examination of Dairy Products (3). To avoid false readings, penase discs confirmed the presence or absence of penicillin. This method was sensitive to at least 0.05 I.U./ml.

As might be expected, the milk from infused quarters (aqueous and oil carriers) showed large zones of inhibition through 48 hr. These data (in Table 1) follow a pattern similar to that reported in previous work in cows from this station (1, 2). When oil suspension was used, milk from the infused quarter of half the cows had detectable quantities of penicillin for as long as 96 hr. At 60 hr. post-infusion, the milk of one cow given penicillin in aqueous solution contained penicillin. When the goat experiments were repeated in early lactation (72 days in milk) rather than late lactation (246 days post-partum), the antibiotic persisted for 98 and 108 hr., respectively. This agrees with the work of Jackson and Bryan, who found that the highest penicillin levels in cows were attained near the middle or end of lactation (4).

TABLE 1

Presence of penicillin in milk following infusion [a]

Doe No.	Hours post-administration												
	0	12	24	36	48	60	72	84	96	108	120	132	144
1. Oil suspension													
211	−[b]	+[c]	+	+	+	+	+	+	+	+	+	−	−
212	−	+	+	+	+	+	+	+	+	+	+	+	−
239	−	+	+	+	+	+	+	+	+	−	−	−	−
260	−	+	+	+	+	+	+	+	+	+	+	−	−
268	−	+	+	+	+	+	+	+	−	−	−	−	−
232	−	+	+	+	+	+	+	+	−	−	−	−	−
251	−	+	+	+	+	+	+	+	−	−	−	−	−
261	−	+	+	+	+	+	+	+	+	+	+	−	−
263	−	+	+	+	+	+	+	+	−	−	−	−	−
254	−	+	+	+	+	+	+	+	−	−	−	−	−
2. Aqueous solution													
211	−	+	+	+	+	+	−	−	−	−	−	−	−
212	−	+	+	+	+	+	−	−	−	−	−	−	−
239	−	+	+	+	+	−	−	−	−	−	−	−	−
260	−	+	+	+	+	+	−	−	−	−	−	−	−
268	−	+	+	+	+	−	−	−	−	−	−	−	−
232	−	+	+	+	+	−	−	−	−	−	−	−	−
251	−	+	+	+	+	+	−	−	−	−	−	−	−
261	−	+	+	+	+	+	−	−	−	−	−	−	−
263	−	+	+	+	+	−	−	−	−	−	−	−	−
254	−	+	+	+	+	+	−	−	−	−	−	−	−

[a] In the treated half. All untreated halves were negative for the presence of penicillin.
[b] Denotes absence of penicillin.
[c] Denotes presence of penicillin at the level of $>.05$ unit/ml of milk.

Data in Table 1 indicate that the antibiotic persisted longer in goats than in dairy cows (1). It should be recognized that when penicillin is the treatment of choice, the routine dosage level for dairy goats and cows is generally the same, or 100,000 units of penicillin. The therapeutic dosage level for the goat is essentially ten times higher than for the cow.

The younger does (first lactation) retained the infused penicillin for a shorter time than the older does (second through fifth lactation). At 72 hr. post-infusion seven of 11 younger does and nine of nine older does were positive for penicillin. At 120 hr. post-infusion, two of 11 younger does and six of nine older does were positive for penicillin.

When 20 does were checked in early and late lactation, for the transfer of penicillin from the infused to the uninfused side, there was no measurable transfer of the antibiotic. Some recent studies in the cow, using highly sensitive methods, indicate that antibiotics are transferred from treated to untreated quarters, but with penicillin this transfer is slight and of short duration and not likely to present a problem (2).

Although penicillin was not detected in the noninfused half, it was found in the blood for 12 hr. post-infusion in seven of 20 does in the early stages of lactation. In late lactation following infusion no penicillin was detected.

The relatively short persistence and nontransfer of penicillin in the mammary gland of the dairy goat, as measured at a sensitivity level of 0.05 I.U/ml, emphasized a basic difference between the mammary gland of the goat and of the cow. Following infusion of penicillin and its carrier into the udder of a goat, a considerable portion of the material would remain in the teat and gland cistern regions, which are relatively much larger than in the cow. In addition, the goat udder is somewhat funnel-shaped, with more complete separation, distance, and integrity between the halves.

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SYMPOSIUM: A MODERN DAIRY CATTLE FEEDING PROGRAM[1]

RESULTS OF FEED ANALYSIS IN FEEDING DAIRY CATTLE

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Since the beginning of the Penn State Forage Testing Service in January, 1959, through May 31, 1961, 1,377 users have submitted 4,293 samples of hay, silage, and grain. The operational details of this program have been described elsewhere (1, 4). This service might be more properly called a feed-programming service, inasmuch as 84% of its users have requested feeding programs for dairy cattle or other livestock.

SURVEY OF USERS

Some individual case histories relating the benefits of this program to dairymen have been published previously (2). Similar success stories have been called to our attention by many more individuals. We desired, however, to ascertain the effects of using this service on a much broader basis, in order to more fully evaluate the program. Thus, a detailed study was made of all users of the service in 13 counties. These counties were among those having considerable success from the standpoint of number of participants.

A partial summary of the user survey is presented in Table 1. Several criteria indicate that the responding users were representative of all users in these counties and the state. The 40% return was considered excellent, in view of the survey being conducted by mail. Apparently, there has been a high rate of adoption of most suggestions on the feeding of the milking herd. This is noteworthy, since most of the suggestions made regarding grain mixtures, protein levels as well as dry and fresh cow feeding, have represented a rather drastic departure from previous practice on many of these farms. A lower rate of adoption has occurred for suggestions relating to the feeding of young calves and heifers over 4 mo. of age. Over 80% of the users indicated that testing has helped them to improve forage quality. Most of the factors listed as being responsible may be categorized, as follows: (1) more selective feeding of available forages; (2) ear-

[1] Presented at the A.D.S.A. Annual Meeting, University of Wisconsin, Madison, June 13, 1961.

TABLE 1

Survey of individuals using forage-testing service[a]

Questions	Per cent indicating	
	Yes	No
	(%)	(%)
1. Did you request a feeding program?	84.1	15.9
2. What feeding suggestions, if any, were followed?		
(a) Per cent protein in grain mix for cows	91.3	8.7
(b) Grain-to-milk ratios	91.8	8.2
(c) Heifer feeding	58.8	41.2
(d) Calf feeding	43.1	56.9
(e) Amounts of grain for dry cows	88.5	11.5
(f) Fresh cow–feeding schedule	88.8	11.2
(g) Grain mixture for cows	82.0	18.0
(h) Grain mixture for calves	36.7	63.3
(i) Was the feeding of 2-3 lb. of good alfalfa recommended to improve feed utilization?	50.5	49.5
(j) If answer to (i) is yes, was this followed?	56.1	43.9
3. Has what you learned from forage testing helped you to improve forage quality?	80.4	19.6
4. Has forage testing helped you to reduce the cost of your grain mix?	61.7	38.3
5. Has forage testing helped to improve herd health?	54.0	46.0
6. Has forage testing helped to increase production levels?	72.3	27.7
7. Has forage testing helped to increase profits on your farm?	81.6	18.4
8. Do you need further help in interpreting and using the information provided in your reports?	22.0	78.0
9. Would you recommend the forage-testing service to others?	96.7	3.3

[a] Replies received from 241 of 604 users surveyed in 13 counties.

lier cutting; (3) sounder choice of annuals for silage; and (4) improved methods for making hay-crop silage.

The cost of the grain mix was reduced in 62% of cases responding to the survey. Herd health apparently was improved on 54% of the farms. More than 72% of the users claimed that forage testing helped them to increase production levels. An even higher proportion (82%) indicated that it had helped to increase profits on their farms. Some phase of feed programming was mentioned in 62.5% of the cases as being most helpful, whereas some aspect of forage evaluation was listed by 37.5% of those responding. Among the feeding phases listed as being most helpful, the dry and fresh cow feeding schedule was cited most frequently. Reduced feed cost followed closely. Most of the points raised by the 22% of the users desiring further help were related to forage evaluation and methods by which quality might be improved. Almost 97% of the users would recommend the service to others. Of those who would not recommend it, about 50% had not requested feeding programs. Two users complained that the cost of $5 per sample was too high. One stated that he would not recommend the service to others because it would weaken his competitive advantage.

BEFORE AND AFTER STUDY

In addition to the survey, a study was made of the DHIA records of users in these 13 counties. Approximately 65% of the users of the service in the state are enrolled in the standard DHIA or Owner-Sampler programs. The results of the before and after study may be found in Table 2. Improvement in milk fat production in herds of users was 55% greater than for all DHIA herds in the same counties. Despite the increase in milk production, milk fat test increased for both groups of forage-testing users, compared to no change for all DHIA herds. No appreciable change in amounts of grain fed occurred for users compared to an increase of over 200 lb. per cow in all herds. Cost of the grain mixture decreased more for all herds than for users. Hay was fed heavier by all groups. Silage feeding was reduced to a considerably greater extent by users than by all DHIA members.

Feed costs dropped $2.66 per cow for all users, compared to an increase of $5.75 in all DHIA herds. This decrease in feed costs occurred in face of greater milk production. No appreciable change in feed costs resulted in herds of users having increased milk production. Return over feed costs increased nearly three times as much for all users compared to all DHIA members. The improved return for users with a production increase was almost six times that for all herds on test in the 13 counties. The data for users with a production increase were included merely to provide a more adequate reflection of the potential of this program for dairymen. The 68% of users having a production increase according to DHIA records compares favorably with the 72% reporting an increase in the survey of all users.

Actually, the improved returns in both groups of users are a conservative estimate of the gains to dairymen. Our DHIA testing year begins on October 1, and a majority of the comparisons were from herds not using the service until late January through April. Thus, any effects would be attained over less than a full testing year. Also, we know from our survey that at least 10% of the users did not follow the feeding suggestions. No benefits from feeding of young stock are reflected in Table 2.

TABLE 2
Influence of forage testing[a]

	Yearly change		
	All users	All DHIA	Users with production increase
Milk production *(lb/cow)*	+370	+269	+849
Milk fat test *(%)*	+0.02	No chg.	+0.01
Milk fat *(lb/cow)*	+ 17	+ 11	+ 34
Grain fed *(lb/cow)*	− 7	+211	+ 20
Cost of grain/ton	−$2.20	−$2.80	−$1.20
Hay fed *(lb/cow)*	+268	+241	+206
Silage fed *(lb/cow)*	−370	− 41	−297
Pasture days/cow	− 2	+ 6	− 1
Feed costs/cow	−$2.66	+$5.75	+$ .18
Return over feed costs/cow	+$20.79	+$7.43	+$41.42
No. comparisons	173	1,309	117
Before milk prod. *(lb/cow)*	10,562	10,475	10,097

[a] Based on DHIA data from 13 Pennsylvania counties. All data were adjusted to common milk and feed prices, except for cost of grain. Only records from year previous to using forage testing and the first year after using were compared.

The before-and-after comparisons were based on the first year after use of the forage-testing service. Previous surveys of two counties suggested that only 50-70% of the users tried many of the feeding suggestions during their first year of participation. The satisfactory results obtained by neighbors, demonstration farmers, and other educational activities encouraged them to make better use of the feeding information during the second year. The adoption rate of first-time users apparently has risen considerably in subsequent years, for similar reasons. Numerous herds in the study did not make any appreciable improvement until their second year of forage testing. In many other herds, healthy increases have occurred each year. Comparisons beyond the first year of forage testing were not included in the data given in Table 2.

The average yearly cost of forage testing at $5 per sample has been $13 per herd. Allowing for the increase in return for all DHIA herds, the adjusted increase in return over feed costs for one cow ($20.79—7.43 = $13.36) paid the forage-testing bill for the entire herd of the average user. The benefits derived from the program appear to be largely a function of the effectiveness with which the suggestions are implemented. Various factors indicate that only 5-10% of our dairymen can not benefit appreciably, if the program is used properly. Looking at some of the more notable results in individual herds, milk production has been increased as high as 4,300 lb. per cow. Grain costs have been reduced as much as $25 per ton for some dairymen. Some dairymen have fed profitably 1,000-2,700 lb. more grain per cow. Returns over feed cost have been boosted as high as $134 per cow. The average increase in returns has been $36 per cow in demonstration farm herds. Numerous beef, sheep, and swine feeders also have benefited greatly from using the forage-testing service.

PARTICIPATION BY FEED TRADE AND OTHER GROUPS

Until the fall of 1960, no active attempt was made to encourage participation by the feed trade. Since its beginning, approximately 23 feed concerns or outlets have actively used our program in servicing their accounts. At least 29 more concerns or outlets have participated to a lesser extent. A breakdown of the types of grain mixtures suggested by our forage-testing service is given in Table 3. A manufactured dairy feed or concentrate was suggested in 51% of the cases. These data were based on the programs developed during the first 2 yr. of operation. There appears to have been an increase in complete dairy feeds suggested during the past season. This stems from a rather distinct trend towards bulk purchasing of feed and sizable quantity discounts. In studying the data in Table 3, it should be kept in mind that a reasonably adequate supply of farm grains for dairy cattle feeding is available in about 70-75% of the areas in Pennsylvania. The suggestions were made on the basis of feed supply and price data submitted by the individual user. Generally, the choice resulted from price considerations, while maintaining a nutritionally adequate ration. In some cases, the user specifically requested certain ingredients or did not provide price information to allow for a choice. We are confident that an even greater proportion of manufactured feeds would be suggested if the feed trade were playing a more active role with the users of the service. Our program has been set up in a manner which enables active participation by all members of the feed trade. The majority of those who have actively participated to date find that it is one of their most useful service and sales tools. Thus, participation by the feed trade and other groups is increasing. We want to encourage such, for reasons detailed later. About 62% of the participating feed concerns are independent organizations. Other groups actively using the service include FHA supervisors, vocational agriculture personnel, DHIA supervisors, and other agricultural trade people. Many of our promotional efforts will be aimed at these people, who are in direct contact with farmers.

TABLE 3

Distribution of grain mixtures suggested

Type	Frequency
	(%)
Grains [a] + oil meal	49
Groins [a] + manufactured concentrate [b]	30
Manufactured dairy feed [c]	21

[a] Include by-product ingredients in some cases as well as molasses and minerals in most mixtures.
[b] Example: 32-50% concentrate.
[c] Example: 16% dairy ration.

OBJECTIVES OF THE SERVICE

The forage-testing service conducted in Pennsylvania is not a panacea for feeding dairy cattle. This program was designed with certain objectives in mind, as follows:

(1) To provide a detailed and personalized service for farmers, who desire a feeding program based on the best information currently available.
(2) To demonstrate and hasten the adoption of sound feeding practices taught in our other extension activities.
(3) To provide people who are working directly with farmers with another means of helping farmers in this era calling for more precise management.

After 3 yr. of experience, we definitely feel that this farm-tested program meets these ob-

jectives. Actually, this program resulted from demands by leading farmers for a feed-programming service that went beyond anything available to them in the past. One can not rely on this service alone to provide farmers with feeding programs or to conduct extension education in dairy cattle and livestock feeding. Dairymen, like other people, vary in their managerial abilities and goals in life. Thus, it is important to have programs that will meet the needs of people belonging to as many of the basic categories as possible. Our forage-testing program meets the needs of the farmer with good managerial ability and the one who demands this type of detailed and personalized service. It also is extremely valuable in working with problem herd situations at all rungs in the ability ladder.

Our conventional type of program is based largely on descriptions of forage quality, average analyses for various qualities and types of forage, and indirectly on the Cornell date-of-cutting principle. It includes various publications and teachings related to grain-feeding tables, mechanics of feeding, dry and fresh cow feeding, and grain mixtures. Considerations are being given to expanding this conventional approach to include grain-feeding recommendations for individual cows in our DHIA program. This might be based on the TDN content of various forages as determined by forage analysis, date of cutting, or average analyses for the types of forage involved. The latter would be based largely on the results obtained from our forage-testing program. This conventional approach undoubtedly is suited to the dairyman who is not ready for the more precise and detailed forage-testing program. Perhaps he does not have the ability to utilize the forage-testing information, or his goals are not sufficiently high to interest him in using a more detailed program. The conventional-type program allows for the individual approach to feeding dairy cows. This also is true of the forage-testing program.

There are a large number of dairy farmers who need a simplified approach to dairy cattle feeding. In some cases, this need stems from lack of managerial ability and cowmanship. Some have not been sufficiently squeezed financially to be interested in more complex methods. In a few cases, a simplified approach is deemed expedient by the farmer, because of a large herd-size and labor problems. Such a situation appears to justify a one-shot method of feeding dairy cows, in which the individual cow approach is abandoned, with the exception of a minimum of health, flesh, and appetite considerations. We have tried this to a limited extent on some of our demonstration farms and in some problem herds throughout the state. It has proved quite successful to date. In some cases it helped interest the farmers in their potential dairy income and up-graded them to a more complex program. Under many conditions this across-the board approach to feeding a herd or groups within a herd can improve incomes considerably. We plan to make this alternative program available for these people in the future. It could be developed with or without use of the forage-testing service.

REASONS FOR FORAGE TESTING

Perhaps some people can not see the justification for having a program such as forage testing in addition to a conventional one already described and in general use in many other states. Here are some of our reasons: We feel that providing a guide to amounts of grain to be fed is inadequate in itself in helping the dairyman who wants to do an efficient job. While this can be extremely helpful and is necessary, tailor-made and more precise suggestions on level of protein for the grain mix, low-cost grain mixtures, mineral supplementation, utilization of forages, forage-feeding rates, as well as dry and fresh cow feeding, are important phases of dairy cattle nutrition.

Rule-of-thumb procedures for determining the protein level for the grain mix are many times an improvement over wild guessing. However, they may result in considerable over- or underfeeding of protein. This is pointed out in Table 4. All cows were fed the same ration from the standpoint of forage types, except as noted otherwise. Due to differences in rate of forage feeding, the protein content of the forage, and grain-feeding rates, the crude protein needed in the grain mix varied considerably.

The protein content of any particular type of forage as classified by farmers or others may vary tremendously, as shown in Table 5. Most Pennsylvania forages may be classified as mixed. These complicating factors make it rather difficult to pinpoint the amount of protein required in the grain mix with less complex methods than those used by our forage-testing service. The crude protein contents

TABLE 4

Protein needs of cows fed hay and corn silage

Herd	Av. daily milk production	Corn silage	Hay [a]	Suggested crude protein in grain
		(lb.)		*(%)*
A	46	30	27	16
B	32	24	24	17
C	33	28	15	18
D	44	50	14	20
E	37	35	18	13
F	31	37	18	15
G	30	86	5	32

[a] Grass hay was fed to Herds A, B, C, D. Mixed hay was used in Herds E, F, and G.

TABLE 5

Crude protein content of various types of first-cutting hay

Type of hay	Crude protein content (dry matter basis)	
	Average	Range
	(%)	
Legume	16.4	9.7-20.5
Mixed, mainly legume	13.6	6.4-22.6
Mixed, mainly grass	10.9	6.3-19.5
Grass	9.6	4.7-17.9

suggested by the service to date have varied from 12-36%. Some of the most consistent responses to the feeding suggestions have been in herds which have boosted protein levels to as high as 18-20% after forage testing. An average protein content of 14% has been suggested, with 58% of the suggested programs calling for a 12% mix.

The moisture content of silage may be an important source of error when feeding value is estimated by means other than analysis. A range of 70-89% moisture or greater has been noted for direct-cut, hay-crop silage. Even a more consistent forage, corn silage, has varied from 62-88% moisture. Such a range in moisture alone could result in corn silages varying from 5-28% TDN on a fresh basis. A difference of 5% in the moisture level of hay-crop silage alters TDN content by about 22%.

A wide range exists in the estimated TDN or ENE content of forages under farm conditions. TDN values calculated from crude protein and crude fiber contents may be found in Table 6. The relationships used in calculating these values are as follows:

(1) $DP = 0.946X - 3.52$

Where X = crude protein as per cent of dry matter, and DP = per cent digestible protein.

(2) $ME = 3{,}240 + 14\,X_1 - 39.1\,X_2$

Where ME = metabolizable energy in Calories per kg. of dry matter; X_1 = digestible protein as per cent of dry matter; and X_2 = crude fiber as per cent of dry matter.

(3) $$\text{TDN} = \frac{\text{Calories of ME per kg. dry matter}}{3{,}563\ (\text{Calories ME per kg. TDN})} \times 100$$

When TDN = per cent total digestible nutrients.

Unless otherwise noted, these relationships have been used in computing the calculated values presented in this paper.

The use of average analyses or expectancies can be of limited value only in feed programming, especially if based largely on research conducted under controlled conditions. The use

TABLE 6

TDN[a] content of various forages

Forage	TDN content (dry matter basis)	
	Average	Range
Silages:	(%)	
Hay-crop	55.3	40.5-73.5
Corn	63.9	43.4-74.3
Other annuals	55.1	39.3-69.6
Legume, mixed mainly legume hays		
First-cutting	57.7	44.1-70.2
Aftermath-cutting	61.5	47.4-76.8
Grass, mixed mainly grass hays		
First-cutting	54.3	39.1-70.4
Aftermath-cutting	59.8	51.5-66.6

[a] Calculated values.

of the date-of-cutting principle can help to more accurately pinpoint nutritive content. Chemical analysis and artificial rumen techniques also can be of benefit in estimating nutritive value. Undoubtedly, none of the present methods is infallible.

All of us realize that the nutritive value of hay-crop forage is closely related to date of cutting and stage of maturity. This undoubtedly is the biggest single factor influencing the feeding value of forages. To date the bulk of the published data available applies to first-cutting material. In two-cutting areas, about 80% of the hay-crop forage may result from first cuttings. However, in three-cutting areas about 57% is derived from aftermath cuttings. When the various annual forages also are included, the relative importance of first-cutting forage dwindles even further. The distribution of forage samples in these various categories after 2 yr. of forage testing is given in Table 7. Less than 56% of the samples consisted of first-cutting forage. These data do not necessarily represent the proportions in which these forages were consumed. However, they do indicate that more information is needed before one can go all-out on the date-of-cutting principle.

There are still some indications that species differences may exist regarding the relationship of date of cutting to nutritive value. This

TABLE 7

Distribution of samples tested

Forage	Per cent of total samples
Hay-crop	
First-cutting	55.6
Aftermath-cutting	17.9
Annual forages	26.5

is suggested in the data presented in Table 8. These data are based on research conducted by Bratzler and associates at the Pennsylvania Station in recent years. The digestible energy content of orchard grass was approximately 5% lower than a legume-grass mixture cut on the same date. A bird's-foot-trefoil–orchard grass mixture apparently decreased in digestibility at a considerably slower rate than alfalfa–orchard grass forage. On any given date, forage intake was greater for the bird's-foot–orchard grass mixture. Thus, the nutritive value index (NVI) was greater and decreased at a much slower rate for the bird's-foot-trefoil-containing forage. In Study I, dry matter intake also was considerably higher for the legume-grass mixture at any given date or stage of maturity. The research, from which the data in Study I were obtained, has been published elsewhere (6).

Data obtained from forage plots harvested at four widely separated locations indicate that species differences comparable to those reported for digestible energy occur among calculated TDN values for forages cut on the same date. Slight or negative differences in nutritive content were noted among varieties. This is in general agreement with the Cornell findings. While species differences apparently existed, the rate of decline in digestibility for the average of all species was strikingly similar to that reported by the Cornell workers. Absolute values and rate of decline were quite similar for all locations, with the exception of Southeastern Pennsylvania. A possible exception to the varietal differences was Dupuits alfalfa, which averaged about 4% lower in TDN content than Vernal for all harvest dates.

While the averages for all forages harvested on small plot basis closely resembled the Cornell work, data obtained from larger numbers of forages harvested under farm conditions did not appear to follow a similar pattern (Table 9). Although they are by no means conclusive, these data suggest that factors other than cutting time may exert considerable influence on the nutritive content of forages. It appears from these data and results obtained from our unit demonstration farms that problems exist in making good-quality hay-crop silage under field conditions. The data also suggest that date of cutting may not be as accurate for silages from the field as for hay. Since the calculated TDN values of these silages were lowered primarily by their relatively high fiber content in spite of early cutting, it was suspected that the formulas used may be grossly inaccurate for the early-harvested silage.

Four lots of problem silage were obtained from farms throughout the state, to test this hypothesis on a limited scale. Neither the date of cutting nor forage-testing procedures provided an accurate evaluation of two of these silages. Date of cutting was somewhat more accurate for the remaining two silages. These silages were characterized as problem ones on the basis of their high fiber content in relation to date of cutting. A comparison of the fiber content of the forage as it was ensiled with the values for the silage when fed, indicated that an increase of as much as 6-8% occurred during ensiling on these farms. Further digestion trials on silages made under farm conditions are contemplated.

TABLE 8

Influence of cutting date of the nutritive value of first-cutting forage

Date cut	Study I Digestible energy: Alfalfa–clover timothy mix	Study I Digestible energy: Orchard grass
	(%)	
May 25	69.2	63.8
June 9	62.6	.....
June 24	58.3	53.4

	Study II: Alfalfa–orchard mix	Study II: Bird's-foot-trefoil orchard mix
June 5		
Digestible energy	69.2%	66.6%
NVI [a]	82.0	93.0
June 15		
Digestible energy	58.2%	64.3%
NVI [a]	71.0	89.0

[a] Crampton's Nutritive Value Index (5).

TABLE 9

Influence of date of cutting on TDN [a] content of hay-crop forages

Interval	TDN content (dry matter basis): Hay	Silage	Combined forages	Research plots
	(%)			
May 1-20	61.0	57.6	59.5	.....
May 15	.....	.....	.....	68.7
May 21-31	60.3	56.2	56.9	.....
May 29	.....	.....	.....	60.3
June 1-10	57.5	54.7	55.9	.....
June 11-15	56.0	54.6	55.4	.....
June 12	.....	.....	.....	55.5
June 16-20	56.2	.....	56.2	.....
June 21-30	55.0	55.0	55.0	.....

[a] Calculated values.

A summary of forage evaluation results suggests that considerable differences also exist among some methods of ensiling hay-crop forages under farm conditions. These data have been published recently (3). There also are indications that some haying methods influence the nutritive content of hays. Most of these differences obtained with forage-testing samples approximate those reported by Beltsville workers. Strict use of date of cutting in forage evaluation largely ignores differences in nutritive content, due to methods used. This may make it more difficult for farmers using the more expensive methods to minimize losses or maximize profits, whichever the case might be. This may be especially true in areas where little or no difference exists in the cost of nutrients in the form of forage or grain.

This discussion of evaluation has been included not to degrade present methods but to emphasize that further research is needed to improve upon them. This need for improvement should not be a deterrent to using present methods, whether it is date of cutting, chemical analysis, or some other means. The gains which can be made even today are too great to justify further postponement of service to farmers in feed programming.

Results obtained on some unit demonstration farms and in numerous other herds throughout the state indicate that the fresh-cow feeding schedules developed for users of forage testing have been largely responsible for an increase in milk production of 1,200-3,300 lb. per cow, with a corresponding increase of only 200-800 lb. of grain for the entire year. In some cases, no increase in grain feeding has occurred on a yearly basis. To a large extent, these schedules result in shifting heavier grain feeding to a period immediately before and after parturition. A reduced incidence of metabolic disorders has occurred in many herds in which these schedules are closely followed. One strong feature of the fresh-cow feeding guide is a matter of feeding according to potential—not necessarily according to past or present production levels. Unless production response requires heavier feeding, cows are fed grain at a constant level until it is clearly evident that they have peaked for the lactation.

Suggestions regarding amounts of forage fed also are given. These suggestions deal with economic considerations in herds purchasing forage, and with intake and quality in some milk fat test problem herds. Other suggestions on forage feeding, especially for dry cows, are often made. Special considerations are given and suggestions made for herds troubled with specific problems. This type of service is difficult to program for electronic processing.

DISADVANTAGES OF FORAGE TESTING

Almost any type of program has disadvantages of one kind or another. Forage testing and feed programming, as it is conducted at Penn State, is no exception. Some of these disadvantages apply to use by the farmer, while others are operational in nature.

(1) *Limited participation.* Even under the best of conditions, probably not over 20-25% of the farmers would be sufficiently interested to use this type of service. Some operational problems presently are limiting participation. First, county agents must be convinced of the value of the program. This is easier to do after several years of experience than at the beginning. County personnel need help in organizing their program to accommodate and service forage testing. A majority of county personnel still need convincing and further help in organizing the program. Much of this lag stems from insufficient effort on our part. This phase of our work will receive greater attention in the future. Experience has shown that good participation can not be obtained with mass media methods. Personal contact, as well as help with sampling and interpretation, is needed to obtain a satisfied user. This means that DHIA supervisors, feed trade, fieldmen for various organizations, and other governmental agencies must be encouraged to take an active role. This is especially true from the standpoint of making forage samplers, cartons, and forms readily accessible to farmers. To date only 34% of our users are repeat customers from one year to the next, despite the fact that 97% of them consider it as an extremely valuable program. According to our survey, the biggest detriment to repeat use is that supplies are not readily available. Where this problem is alleviated, 60-70% of the users repeat from one year to the next.

(2) *Processing period.* An average of 16-17 days has been required from the time samples and forms are mailed by the farmer until he receives his reports. While this has not materially reduced the value of the program, it is a justifiable complaint. Using present methods, this period probably could be reduced by three to five days, if analyses were performed at the University rather than at a commercial lab located at a distance. Five to eight days are required for mailing time alone.

(3) *Personnel requirements.* An equivalent of 50-60% of the time of one specialist is needed to operate the program during 6 mo. of the year. A smaller percentage of time is required during the off-season. This means that other projects or areas of work must suffer when staff size is limited for project work. Clerical or technical help requirements and supplies may be met if approximately $1.50 to $2 is charged per sample over analytical costs.

ADVANTAGES OF PROGRAM

Some of these help to counteract the disadvantages already discussed. The following advantages are derived from the program as it is conducted at Penn State:

(1) *Increases adoption of sound practices.* Not only do the users of the service benefit, but their neighbors adopt many of the practices common to most farms and recommended in our other educational programs. For example, forage testing, coupled with the unit demonstration farm program, undoubtedly has played a major role in reducing the costs of the grain mix in DHIA herds an average of $5.60 per ton over the past 2 yr.

(2) *Discourages reliance by farmers on cure-alls.* This should be readily understood by people who realize the great pressures placed on farmers today.

(3) *Strengthens extension and research programs.* The organizational aspects of our program require close cooperation of both research and extension personnel in agronomy, nutrition, and allied disciplines. This environment in itself has improved effectiveness in other phases of work. Both extension and research personnel are kept better-informed of needs in the field. Numerous changes in extension teaching and research approaches already have resulted. Some farmers who have not been considered regular extension cooperators in the past are using the forage-testing service. These include innovators and some non-adopters who now are searching rather desperately for crutches to ease their plight.

(4) *Helps to standardize recommendations.* An active program of this type encourages feed-trade people and other organizations, including ourselves, to standardize our teachings and programs wherever possible. This encourages adoption of information by farmers.

(5) *Provides extension personnel and others with a means of improving service to farmers.* County workers can not be specialists in all phases of agriculture and even feed-trade personnel can not be specialists in the nutrition of all livestock. The needs of the farmer in this complex business of agriculture are for detailed service and information. Forage testing helps to meet the needs of all concerned.

(6) *Enables feeding suggestions to be tailored to conditions on the individual farm.*

(7) *Provides a means of keeping feeding programs current with latest information from research and farm testing.* The lag between gaining new or improved knowledge and its adoption by farmers is too great.

(8) *Fits many needs.* Our forage-testing service is a program which can be of value to the small farmer as well as the large, with high forage or high grain feeding; to the feed dealer with an extensive field service program or the one without any; to the veterinarian or the fertilizer dealer; to the DHIA member or the nonmember; and to the sheep feeder as well as the dairymen.

As noted earlier, many of the benefits derived are associated with feeding programs. It is doubtful that forage analysis alone would be of much value if help is not rendered in some manner in translating such information into sound feeding practices. Our forage-testing service is not the only course that should be taken helping farmers with feeding programs. Also, there is plenty of room for improving it. This we hope to accomplish as more experience is gained in Pennsylvania and elsewhere, and research enables the use of better methods. We believe that our program has been and will continue to be of great value. Undoubtedly, it is but one step towards providing a more complete service to help farmers with decision-making. We are spending an increasing amount of effort towards the development of a program which will consider the entire farming operation.

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HIGH-LEVEL GRAIN FEEDING FOR DAIRY COWS

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Our interest in the more liberal grain feeding of good cows was prompted by the field observations of Donald Hillman of the MSU Dairy Department, noting that many inherently high milk producing cows were underfed on energy. This is the case, despite the fact that the revolution in corn production, due to the use of hybrid corn and better fertilizer and cultural practices, has made possible cheaper sources of nutrients than roughages on many farms.

Lassiter (22) has calculated the relative yields and cost of TDN in some common feedstuffs, as shown in Table 1.

A part of the failure to feed sufficient grain was due to insufficient grain consumption in the milking parlor. Also, many dairymen are reluctant to feed high levels of grain because of the belief that such a practice aggravates mastitis and udder edema and tends to produce off-feed. They are also concerned about the possibility of feeding more grain than is profitable, because of the belief in the law of diminishing returns which applies to poor producers.

In this report some of the road blocks to higher grain feeding to good cows will be briefly reviewed.

Eckles defined a good cow as one capable of consuming a large quantity of feed in excess of her maintenance requirement and of using this feed for milk production.

EFFECT OF HIGH GRAIN FEEDING ON HEALTH

Mastitis

High protein concentrates. Moore (27) of Mississippi, in 1914, reported that the feeding of 5 lb. of cottonseed meal per cow daily in a lot of five cows, produced more garget than in the controls. Most of the investigations with high protein concentrate feeding have failed to incriminate high protein as a factor in causing mastitis. Huffman and Moore (16) fed five cows cottonseed meal and five cows linseed meal at levels of 5 to 12 lb. per cow daily over a 6-mo. period, using bacterial counts on incubated plates, and failed to show that these protein concentrates aggravated mastitis.

Huffman and Moore fed up to 17 lb. of 43% protein cottonseed meal per day to cows at the peak of lactation. Usually, when cows were consuming 17 lb. of cottonseed meal, they were also fed about 13 lb. of corn daily. These data are shown in Table 2.

Similar results were reported by Kuhlman (20) of Oklahoma, who fed 6 to 10 lb. of cottonseed meal per cow daily.

Hotis *et al.* (14) used cows with a history of mastitis. They concluded that the feeding of cottonseed meal (5 to 10 lb. per cow daily) did not aggravate udder conditions or force cows subject to chronic attacks of mastitis into clinical cases.

Although Frost (7) claimed that when protein feeds were fed to slightly infected herds many of the animals soon came down with mastitis, he was unable to produce mastitis in a cow fed cottonseed meal as the only concentrate or in another one fed corn gluten meal.

High grain rations. Moore *et al.* (26) of West Virginia used 20 milking cows fed grain (high corn) at the rate of 1 lb. for each 3.5 lb. of milk. All animals were fed legume hay, corn silage, and pasture in season and managed as a single herd. After continuing the experiment for 2 yr., they concluded, "There appears to be no significant difference in the incidence or severity of mastitis between the group receiving the heavy corn ration and the group fed a normal ration, as fed in this experiment." In further studies these investigators could find no difference in the incidence or severity of mastitis when first-calf heifers were fed the above control rations at the rate of 1 lb. per 3.5 lb. of milk compared to a low level of grain.

Gardner (8), of the University of Illinois, reported that none of the following rations increased the incidence or severity of mastitis: (a) corn grain and corn silage, (b) corn grain, corn silage, and urea, (c) corn grain, corn silage, and alfalfa hay, (d) corn grain, oats, soybean oil meal, and wheat bran. In Rations (a) and (b), 15 to 25 lb. of corn grain was fed daily for about 1 yr., and in Ration (c)

TABLE 1

Total yield of digestible nutrients per acre and cost of TDN per 100 lb. (22)

	Yield/A	Cost/T	TDN per acre	Cost of 100 lb. TDN
			(lb.)	*($)*
Alfalfa-brome hay	3.6T	$20	3,600	2.00
Hay-crop silage	12T	6.33	4,000	1.92
Corn silage	15T	7.00	6,000	1.75
Ear corn	75 bu.	.85	4,000	1.51
Shelled corn	75 bu.	.85	3,350	1.90
Oat grain	60 bu.	.56	1,344	2.57

TABLE 2

Effect of heavy grain (high protein) feeding on health of milking cows[a]

Cow No.	Lact.	Wt.	Average/day grain high month	High month	Average/day for 305 day lact.	Cow No.	Lact.	Wt.	Average/day grain high month	Month	Average/day for lact.
		(lb.)	*(lb.)*		*(lb.)*			*(lb.)*	*(lb.)*		*(lb.)*
G 1	1	901	19.1	2	15.0	G 7	1	866	14.0	4	11.9
	2	1,138	22.3	3	17.3		2	993	23.0	3	15.3
	3	1,178	20.3	2	19.4		3	978	21.0	3	16.9
	4	1,245	24.1	2	18.3		4	1,072	22.9	2	20.0
	5	1,364	29.1	3	24.4			Second generation			
G 3	1	876	23.8	2	20.0	G 15	1	807	14.6	2	11.0
	2	963	23.1	2	15.9		2	946	20.1	2	16.8
	3	1,154	27.6	3	22.8		3	1,167	27.0	4	20.8
	4	1,165	25.9	3	19.4			Third generation			
	5	1,188	30.7	2	21.5						
C 9	1	893	19.0	4	18.7	G 33	1	916	23.0	5	20.0
	2	1,162	22.5	5	17.2		2	1,135	25.6	5	22.1
	3	1,093	29.0	3	20.4		3[b]	1,119	28.4	5	16.8
	4	1,047	28.0	4	20.0		4[c]	1,132	21.5	5	15.7

[a] Unpublished data, Michigan Agricultural Experiment Station.
[b] Alfalfa, corn silage, corn, and cottonseed meal.
[c] Alfalfa, corn silage, and corn.

about 70% of the nutrients came from the corn plant.

In England, MacPherson (24) investigated 24 herds and classified 13 herds as having a low incidence of mastitis and a high plane of nutrition. Herd management also was better.

Charron (5) of GLF studied the effects of higher grain feeding on 48 dairy farms in New York and New Jersey. The results were based on 442 cows paired as equally as possible during a 2-wk. standardization period under normal conditions. During a 10-wk. test period, one cow of each pair was fed the normal amount of feed and the paired mate was increased in concentrates to 2 lb. per 100 lb. of body weight. Clinical examination of the udders and milk of 207 control and test cows showed that, of the cows examined, only 38% showed clinically

TABLE 3

Relative incidence of health problems during the test period reported by supervisor[a]

Problem	Incidence of occurrence		No. of cows		% of cows	
	Control	High T.D.N.	Control	High T.D.N.	Control	High T.D.N.
1. Mastitis	24	14	18	12	8	5
2. Winter dysentery	13	11	13	11	6	5
3. Knee, leg, or hoof injury	6	3	6	3	3	1
4. Teat injury	4	7	4	7	2	3
5. Ketosis, acetonemia	4	8	4	6	2	4
6. Off-feed	3	8	3	8	1	4
7. Scours	2	1	2	1	Less than 1	Less than 1
8. Hardware	2	1	2	1	Less than 1	Less than 1
9. Heat (problem)	2	3	2	3	Less than 1	1
10. Kidney infection	2	0	2	0	Less than 1	0
11. Leaker	1	2	1	2	Less than 1	Less than 1
12. Sick	1	1	1	1	Less than 1	Less than 1

[a] From E. C. Charron. Higher T.D.N. Feeding of Dairy Cows. Cooperative G. L. F. Exchange, Ithaca, New York. 1960.

Notes:

1. Data from a field study involving 442 paired Holstein cows on 48 farms.
2. Controls received regular rate of grain (normally 12-16 lb. per day). High T.D.N. cows—grain was increased to 2% of body weight during the 10-wk. testing period.
3. Cows were examined by local veterinarians at the beginning and end of the trial. Final examination and laboratory test of milk samples for 207 cows showed that higher grain fed cows had 3% more normal udders and 12% less streptococcus infection in clinically normal udders than the control cows.

normal udders at the initial examination. The data did not show that grain feeding resulted in more udder infection, but that the incidence of infection was somewhat reduced on the high grain feeding as compared with the control group (Table 3).

Edema of the udder. Greenhalgh and Gardner (9) reported that feeding grain up to 9 lb. per day per heifer and up to 12 lb. per cow per day for about a 6-wk. period before calving gave no evidence that grain feeding increased edema of the udder. Similar results were obtained by Hemken *et al.* (11). The feeding of 16 to 18 lb. of concentrates per day for 40 to 50 days before calving and until swelling disappeared did not increase the amount of udder edema. Similar results were reported by Hathaway *et al.* (10).

Schmidt and Schultz (32) fed one group of cows no grain, a second group received 6 lb. daily, and a third group received 15 lb. of grain per day during the 8-wk. dry period. The cows were fed 10 lb. of grain on the day after calving and the amount was increased 1 lb. daily until the cows received the amount of grain warranted by their milk production. Heavy grain feeding before and after calving did not appear to increase the severity of udder edema. Similar results were reported by Fountaine (6).

According to Nevens (29), the self-feeding of eight dairy cows for periods of 15-130 wk. indicated that the method is not harmful to the health of cows, provided they are gradually accustomed to it. The 22 cows used in an unlimited grain feeding experiment by Borland *et al.* (2) remained in good health with no more digestive, breeding, or udder troubles than the cows on a low level of grain feeding. The average daily grain consumption was 24.6 lb. through the lactation periods. Some cows readily ate 35 lb. or more of grain daily during the peak of milk production.

According to Jensen *et al.* (18), in their 2-yr. study, udder troubles were more prevalent in the highest fed group, but there was no difference among the five other groups. Udder troubles were not excessive. The level of feeding had no effect on reproductive failures. There was a definite trend upward in the number of cases of off-feed as the level of feeding increased. Very few of these cases were serious. Cows that were fed all the grain they would eat sometimes went off-feed once or twice and, thereafter, avoided overeating.

Results of many experiments show that the occurrence of mastitis and udder edema is not due to heavy grain feeding.

Greater profits above feed cost due to increased grain feeding to good cows. The main objective of cow-testing associations is to enable the dairymen to cull unprofitable cows. Our approach to the effect of higher grain feeding on economic efficiency of milk production is identical with the cow-testing concept.

TABLE 4
Arrangement of data by Ross *et al.* (31)

Group No.	No. of cows	Class	FCM	Total grain	Total hay	TDN in hay	TDN in hay + lb. gr.	FCM lb. feed	Feed cost	Value of milk	Value of milk above feed cost
			(lb.)							*($)*	
1	21	2500-3500	2,896	1,437	2,564	1,282	2,719	1.07	61.57	115.84	54.27
2	79	3500-4500	3,848	1,809	2,651	1,326	3,135	1.23	71.74	153.92	82.18
3	183	4500-5500	4,761	2,122	2,968	1,484	3,606	1.32	82.73	190.44	107.71
4	254	5500-6500	5,670	2,378	3,253	1,627	4,005	1.42	91.98	226.80	134.82
5	309	6500-7500	6,592	2,611	3,397	1,699	4,310	1.53	99.25	263.68	164.43
6	266	7500-8500	7,374	2,870	3,629	1,815	4,685	1.57	108.04	294.96	186.92
7	221	8500-9500	8,284	3,109	3,663	1,832	4,941	1.68	114.36	331.36	217.00
8	137	9500-10500	9,044	3,441	3,715	1,858	5,299	1.71	123.18	361.76	238.58
9	76	10500-11500	9,938	3,698	3,521	1,761	5,459	1.82	127.66	397.52	269.86
10	33	11500-12500	10,689	3,915	3,332	1,666	5,581	1.92	131.20	427.56	296.36
11	19	12500-13500	11,514	4,627	3,842	1,921	6,584	1.76	154.10	460.56	306.46
12	4	13500-14500	12,270	3,568	3,285	1,643	5,211	2.35	122.05	490.80	368.75
13	1	14500-15500	14,000	5,486	3,617	1,809	7,295	1.92	173.32	560.00	386.68

The following prices were used: hay $20/T; grain $50/T; and milk $4 per hundred. The corn grain (14% corn) and hay equivalent in corn silage was estimated by multiplying by 0.13 and 0.20, respectively (14).

To evaluate the effects of increased grain feeding on the economies of milk production, it is necessary to consider the cow as an individual, or arrange the groups of cows on the basis of different levels of milk production. Ross *et al.* (31), of the University of Illinois, in 1923 used such an arrangement as shown in Table 4. These investigators took the results of 15 cow-testing associations and separated the cows according to the level of production, from a low of 2,500 to 3,500 lb. of milk yearly to high production, 16,500-17,500 lb. of milk per year. The authors, as a result of this study, stated "feeding for maximum production is subject to the economic law of increasing costs, while increasing the potential ability for high production is subject to the law of decreasing costs."

The data in Table 5 also illustrate this method from the data supplied to Turner (33) by the Illinois testing plant at Dixon, Illinois. In this table the value of milk above feed costs ranged from $296 at 14,116 lb. to $592.59 at 23,013 lb. of FCM. Concentrate feeding ranged from 8,394 to 10,619 lb. per year. Turner (33) pointed out that the economic law of diminishing returns may operate with average or poor cows that are fed above their requirements, but it did not operate with good producing cows. He found that the milk production per pound of grain increased as the yearly milk production increased.

In an early study, Woodward (35) compared the same cows when yearly milk records were produced under test cow conditions with their performance under ordinary herd conditions. Results are shown in Table 6. The cows showed greater return above feed costs when under ideal conditions than when kept under herd conditions, except Cow 82 for the first lactation as a 2-yr.-old.

TABLE 5

Relation between milk production and feed intake (33)

Cow No.	FCM	Total hay	TDN in hay	Total grain	TDN in forage + lb. grain	FCM lb. feed	Feed cost	Value of milk	Value of milk above feed cost
			(lb.)				($)		
4	14,116	5,877	2,939	8,394	11,333	1.25	268.62	564.64	296.02
10	15,466	5,818	2,909	8,544	11,453	1.35	271.78	618.64	346.86
7	17,283	6,204	3,102	9,585	12,687	1.36	301.67	691.32	389.65
7	18,658	6,072	3,036	9,715	12,751	1.46	303.60	746.32	442.72
10	20,098	6,033	3,017	9,794	12,811	1.57	305.18	803.92	498.74
4	21,459	5,898	2,949	9,544	12,493	1.72	297.58	858.36	560.78
4	23,013	6,245	3,123	10,619	13,742	1.68	327.93	920.52	592.59

TABLE 6

Comparison of herd and test conditions (35)

Cow No.	Weight	FCM	Total grain	TDN	FCM lb. TDN	Value of milk	Feed cost	Value of milk above feed cost
			(lb.)				($)	
50 Test	1,157	14,080	8,112	9,479	1.49	563.74	253.81	309.63
Herd	1,135	11,375	8,171	9,354	1.21	455.00	259.51	240.72
51 Test	1,163	16,065	6,727	8,058	1.99	642.60	232.39	410.22
Herd	1,207	11,466	5,754	6,476	1.74	457.80	189.90	267.90
82 Test	986	10,218	6,367	7,591	1.35	408.72	220.04	188.67
Herd	1,009	10,558	5,599	6,457	1.64	422.32	188.52	233.80
Test	1,226	17,496	9,647	10,225	1.71	699.84	309.84	355.47
88 Herd	970	7,021	3,968	4,530	1.55	280.84	131.92	148.92
Test	1,104	11,892	6,576	7,557	1.57	475.68	220.42	255.26
91 Test	1,172	15,332	7,754	8,807	1.74	613.28	258.04	419.43
Herd	1,247	7,607	4,216	5,854	1.29	304.28	163.43	46.24
94 Test	1,044	13,817	7,718	9,257	1.42	527.48	257.27	269.86
Herd	1,148	10,799	4,977	6,770	1.60	431.96	188.79	243.17
Test	1,310	18,848	9,267	9,820	1.92	753.92	280.49	473.51
299 Test		17,355	8,089	9,544	1.82	694.20	261.98	432.02
Herd	1,212	12,836	6,739	7,146	1.80	513.44	234.29	280.17

Hillman (12, 13) arranged Michigan DHIA data into four classes according to milk production. Results are shown in Table 7.

The grain in corn silage is not accounted for in the DHIA records.

The return above feed cost of highest group (14,727 lb. milk) was $438, compared with the lowest producing group (7,345 lb. milk), $157.

Morrison (28) used records of 29,442 DHIA cows to prepare Table 8. The 5,000-lb. milk group had an $89 return above feed cost, compared with $489 for the group that averaged 17,691 lb. of milk.

Ward, Chestnut, and Huffman (34) assembled MSU data where cows were fed on very low grain, medium grain, and high grain rations in different lactations. A limited amount of these data is presented in Table 9. Cow A6 produced 7,573 lb. of FCM during the third lactation when fed low grain (657 lb.); 10,486 lb. during the fourth lactation when she consumed medium grain (3,547 lb.); and 15,890 lb. during the fifth lactation when 5,862 lb. of grain was fed. Values of milk above feed cost for these three lactations were $302, $419, and $636, respectively. These data suggested that the increased returns above feed cost followed increased grain feeding. Similar results are shown for the other cows used in Table 9.

The principle of diminishing returns as applied to milk production is based primarily on the data by Jensen *et al.* (18). In these investigations large amounts of corn silage were fed. In Table 10 the corn in corn silage was added to the grain fed as such, and the hay equivalent was added to the hay fed as such. In the table, the value of milk above feed cost tended to increase with increased grain feeding. No doubt, if the authors had grouped the cows in each level of feeding according to milk production, the increase in profits would have been much greater for the high-producing cows.

Jensen *et al.* (18), in one of their tables, attempted to group the cows according to the basic producing ability of the cows. The cows from the low stations and from the high stations were grouped into poor cows and good cows. They state that it should be possible to analyze the data by comparing individual cows, while taking full account of the differences in productivity as measured by expected yields based on previous production records, but it is difficult to get such cows for experimental purposes. Jensen *et al.* (18) were of the opinion that for this reason group comparisons are probably more reliable than individual comparisons. This opinion is not in agreement with the concept of Ross *et al.* (31) and Turner (33).

TABLE 7

Michigan DHIA data for 1958. Grain fed at four levels of milk production (12, 13)

Milk production	Grain per cow	Feed cost	Returns above feed cost	Returns per $1 feed cost
(lb.)		*($)*		
14,727	4,703	201	438	3.18
12,689	3,783	170	357	3.10
10,229	2,910	156	269	2.73
7,345	2,154	135	157	2.16

TABLE 8

Relation of level of production to cost of feed and return above feed cost (28)[a]

Av. yield		Value of milk	Cost of concentrates	Total cost of feed	Return above feed cost	Feed cost per 100 lb. of milk
Milk	Fat					
(lb.)				*($)*		
5,073	204	199	55	110	89	2.17
7,390	301	292	69	127	165	1.72
8,545	349	338	76	136	202	1.59
9,640	399	383	83	144	239	1.49
10,784	446	429	89	153	276	1.42
12,048	496	478	98	164	314	1.36
13,276	546	521	103	170	351	1.28
14,150	594	568	112	181	387	1.28
15,486	644	634	120	194	440	1.25
17,085	694	666	128	206	460	1.21
17,691	752	724	150	226	498	1.28

[a] Based on the records of 29,442 DHIA cows that completed a full 12-mo. testing period in 1946.

TABLE 9

Effect of level of grain feeding on efficiency of milk production above feed costs (34)[a]

Cow	Lact.	Weight	Roughage	Conc.	TDN	FCM	FCM lb. TDN	Feed cost	Value of milk	Value of milk above feed cost
				(lb.)					($)	
A4	2	1,143	11,639	2,034	6,629	8,394	1.27	167	336	168
	3	1,240	10,695	3,495	7,667	10,807	1.41	194	432	238
	4	1,257	12,630	2,074	7,705	7,173	.93	178	287	107
A5	3	1,265	11,056	4,706	8,085	11,643	1.44	228	465	237
	4	1,219	13,740	1,937	7,480	10,054	1.34	186	402	216
	5	1,351	10,171	6,154	9,459	15,745	1.66	236	630	374
	6	1,353	13,757	54	6,479	8,026	1.24	139	321	182
	7	1,197	13,030	225	6,643	7,463	1.12	136	299	163
	8	1,245	9,459	4,065	7,416	10,135	1.37	196	405	209
A6	3	1,070	12,528	657	6,191	7,573	1.22	142	302	161
	4	1,027	10,557	3,547	7,747	10,486	1.35	194	419	225
	5	1,231	12,308	5,846	10,087	15,890	1.58	269	636	366
	6	1,207	9,478	3,061	9,228	14,054	1.52	171	562	391
A14	2	1,252	12,314	5,017	9,337	14,689	1.57	249	588	339
	3	1,284	12,262	5,362	9,509	15,324	1.61	257	613	356
	4	1,388	12,347	4,643	8,978	13,752	1.53	240	550	311
	5	1,309	15,082	153	7,983	7,566	.95	165	303	138
	6	1,339	10,527	659	8,334	11,453	1.37	222	458	236
A15	3	1,096	12,733	341	8,735	7,447	.88	136	298	162
	4	1,191	11,095	3,295	7,714	9,993	1.30	193	399	206
	5	1,150	11,417	887	7,968	6,977	1.00	136	279	142
	6	1,256	12,972	4,028	8,849	11,804	1.33	230	472	242

[a] Michigan prices, hay $20/T; corn silage $5/T; grain $50/T; FCM $4/100 lb.

There is a possibility that Jensen *et al.* (18) were not acquainted with these reports.

Further evidence that increased grain feeding does not depress milk production was pointed out by the work of the Delaware station, shown in Table 11. The value of the milk above feed cost tended to increase with the rise in the plane of nutrition. These data were included in the bulletin reported by Jensen *et al.*

Kottke (19) arranged DHIA data according to milk production. As the amount of milk increased, the efficiency per pound of feed increased, as shown in Table 13.

Lindsey and Archibald (23) conducted a long-time experiment with two groups of cows (high and low roughage). The group that received less roughage and more grain produced

TABLE 10

Jensen *et al.* (18). Data from four stations. Cows fed at specified rates of Haecker Standard, Series No. 1 experiments

Item	Unit	Levels 1	2	3	4	5	6
Yearly records	No.	31	28	33	33	16	36
Basic production ability FCM	*(lb.)*	8,836	8,892	8,853	8,841	8,833	8,906
Weight	*(lb.)*	1,127	1,091	1,095	1,136	1,182	1,212
FCM	*(lb.)*	7,558	8,411	8,855	9,999	9,497	11,204
Corn silage	*(lb.)*	12,608	12,499	12,049	12,054	12,197	9,960
Grain in	*(lb.)*	1,639	1,625	1,566	1,567	1,586	1,248
Hay in	*(lb.)*	2,520	2,500	2,410	2,411	2,439	1,920
Grain fed	*(lb.)*	1,876	2,583	3,347	4,469	4,533	6,783
Total grain	*(lb.)*	3,515	4,208	4,913	6,036	6,119	8,031
Hay	*(lb.)*	3,434	3,337	3,422	3,485	3,598	3,678
Total hay	*(lb.)*	5,954	5,837	5,832	5,896	6,037	5,598
TDN in hay	*(lb.)*	2,977	2,919	2,916	2,948	3,019	2,799
TDN in hay and lb. gr.	*(lb.)*	6,492	7,127	7,829	8,984	9,138	10,830
FCM/lb feed	*(%)*	1.16	1.18	1.13	1.11	1.04	1.03
Cost of feed	*($)*	147.42	163.57	181.15	209.86	213.35	256.76
Value of milk	*($)*	302.32	336.44	354.20	399.96	379.88	448.16
Value of milk above feed cost	*($)*	154.90	172.87	173.05	190.10	166.53	191.40

TABLE 11
Data from Delaware Bulletin 248 used in Jensen *et al.* (18) publication

Group	Yearly records	FCM	Total		TDN in hay	TDN in hay + lb. grain	FCM/ lb feed
			Grain	Hay			
			First-year records				
1	6	7,581	3,556	6,411	3,206	6,762	1.12
2	5	8,293	5,046	6,647	3,324	8,370	.99
3	5	8,887	5,716	6,904	3,452	9,168	.97
4	4	11,487	6,552	6,981	3,491	10,043	1.14
			Second-year records				
1	5	8,228	4,495	6,391	3,196	7,691	1.07
2	5	9,844	5,595	6,804	3,402	8,997	1.09
3	4	10,778	6,351	6,514	3,257	9,608	1.12
4	4	12,312	7,867	6,809	3,405	11,272	1.09

more milk per year. The cost of milk production of the two groups was about the same.

Charron (5) reported that milk production increased 2,031 lb. per cow due to higher grain feeding. While 90% of the cows responded with extra milk production, 40% of the cows showed increased profit.

Mather *et al.* (25) found that cows with potential milk production of 8,906 lb. of FCM per 245-day lactation gave 0.58 lb. of FCM per pound of grain; whereas, those with a 245-day potential of 11,708 lb. of FCM gave 1.35 lb. of FCM per pound of grain. The high-potential cows showed no evidence of diminish-

TABLE 12
Different levels of grain with high-quality roughage on efficiency of milk production
New Jersey Agricultural Experiment Station

Group	FCM	Hay equiv.	Hay	Grain	Total TDN	FCM/lb TDN	Feed cost	Value of milk	Value of milk above feed cost	Difference H-O
		(lb.)					($)			
C 1H	Potential	11,741								
	O 8,221	7,339	3,926	0	3,926	2.09	73.39	328.84	255.45	
	M 9,809	6,670	3,549	1,127	4,676	2.10	107.65	392.36	284.71	
	H 14,249	6,606	3,505	2,969	6,484	2.20	173.94	569.96	396.02	140.57
C 2H	Potential	11,726								
	O 7,454	6,514	3,455	0	3,455	2.16	65.14	298.16	233.02	
	M 10,681	6,798	3,599	1,217	4,816	2.22	112.20	427.24	315.04	
	H 13,267	5,742	3,055	3,095	6,150	2.16	169.87	530.68	360.81	127.79
C 3H	Potential	10,832								
	O 8,365	7,157	3,849	0	3,849	2.17	71.57	334.60	263.03	
	M 11,051	6,517	3,450	1,271	4,721	2.34	111.37	442.04	330.67	
	H 12,568	5,968	3,165	2,804	5,969	2.11	161.58	502.72	341.14	78.11
C 4H	Potential	9,412								
	O 8,263	7,510	3,996	0	3,996	2.07	75.10	330.52	255.42	
	M 8,846	6,642	3,534	1,050	4,584	1.93	104.57	353.84	249.27	
	H 11,745	5,850	3,131	2,544	5,675	2.07	150.93	469.80	318.87	63.45
R 5H	Potential	9,803								
	O 8,185	6,849	3,660	0	3,660	2.24	68.49	327.40	258.91	
	M 8,063	6,380	3,383	941	4,324	1.86	98.00	322.52	224.52	
	H 10,789	6,501	3,484	2,353	5,837	1.85	150.51	431.56	281.05	22.14
R 6H	Potential	8,931								
	O 7,139	7,546	4,072	0	4,072	1.75	75.46	285.56	210.10	
	M 8,016	6,980	3,743	1,011	4,754	1.69	106.55	320.64	214.09	
	H 8,811	6,247	3,376	1,973	5,349	1.65	134.17	352.44	218.27	8.17
R 7G	Potential	8,619								
	O 6,909	6,165	3,285	0	3,285	2.10	61.65	276.36	214.71	
	M 9,136	6,260	3,352	1,053	4,405	2.07	100.88	365.44	264.56	
	H 9,774	6,150	3,285	2,345	5,630	1.74	145.70	390.96	244.26	29.55
R 8G	Potential	7,964								
	O 3,733	5,138	2,722	0	2,722	1.37	51.38	149.32	97.94	
	M 5,538	5,197	2,756	652	3,408	1.63	75.67	221.52	145.85	
	H 6,268	4,966	2,641	1,428	4,069	1.54	101.54	250.72	149.18	51.24

[a] This table was supplied by R. E. Mather. J. Dairy Sci., 43: 358. 1960.

TABLE 13
Relationship of herd improvement levels of milk and feed input (19)

Milk average	Composite feed intake (conc. equiv.)	Milk/lb feed intake
	(lb.)	
6,148	6,300	0.98
7,365	8,300	0.89
8,618	7,900	1.09
9,575	8,300	1.15
10,583	8,600	1.23
11,436	9,400	1.22
12,476	9,400	1.33
13,524	10,200	1.33
14,468	11,300	1.28
16,339	14,400	1.44

ing returns and there was relatively little for the low-potential cows.

The Danish government sponsored a 2-yr. experiment with high-producing cows (21). Ten cows with an average production of 607 lb. of fat a year were used. These cows were kept under ideal conditions. Each cow was fed according to her production and appetite. At the peak of milk production as much as 40 lb. of concentrates plus 7 lb. of hay, 30 lb. of grass silage, and 50 lb. of beet top silage were fed. Results are shown in Table 14.

The cows produced an average of 24,341 lb. of FCM during the first year and 25,622 lb. the second year. The efficiency of feed utilization was high compared with average cows in Denmark.

TABLE 14
Feeding of high-yielding dairy cows (21), 365-day lactation records

Cow	4% Fat-corrected milk	Body weight	Increase in weight
		(lb.)	
187	28,142	1,594	99
255	27,282	1,605	174
279	25,796	1,678	148
242	23,697	1,373	64
218	23,735	1,367	79
155	24,954	1,453	181
294	23,446	1,717	134
177	23,660	1,459	179
272	22,582	1,488	190
178	20,097	1,473	309
Average:	24,341	1,521	157
Second year 11 cows averaged:	25,622		77

A day's ration for the highest yield:
52 lb. sugar beets
31 lb. grass silage
7 lb. hay
40 lb. concentrates.

Brody (3) estimated the caloric efficiency for milk production of Carnation Daisy at about 44%. The maximum possible FCM production of dairy cows was estimated at 50,000 lb. per year (3).

In the investigation of the effects of heavy grain feeding upon the efficiency of milk production, it is necessary to use inherently good cows. Many experiments have been conducted using groups of cows of unknown milk-producing potential. Results have been mixed, since it was usually a matter of chance to balance groups in this respect. Recent reviews by Reid (30), Jawetz (17), Burt (4), and Yates (36) cover both the short-time and long-time experiments upon the economics of feeding dairy cows.

It is apparent from this review that good cows with large appetites and inherently high milk production potential are capable of high levels of economic production. It is necessary, however, to treat the data for such cows as individuals. The liberal grain feeding of good cows will make possible the increased profits to dairymen; aid in bull proofs; and aid in the selection of high-potential cows to use in milk-production studies. A testing program is necessary for successful heavy grain feeding.

RECOMMENDED FEEDING RATES
DON HILLMAN

Grain feeding tables and Thumb Rules are, at best, only guides. Adjustments for individual cows and differences in roughage quality are often necessary. The following plan is suggested as a starting point:

Before calving. Feed about 1 lb. of grain for each 100 lb. body weight of the cow for at least 2 wk. prior to calving and continue for four days after calving. A longer prefeeding period may be necessary, depending on the condition of the cow.

After freshening. (a) Continue 1 lb. of grain per 100 lb. body weight for four days. (b) After four days increase grain 1 lb. for each 3 lb. of milk above 30 lb. per day. (c) Continue to increase grain as long as the cow continues to increase in production. When the cow ceases to increase in milk production with increased grain feeding, continue feeding at this level as long as she maintains milk produc-

TABLE 15
Grain feeding schedule

Milk/ day	Grain/ day	Milk/ day	Grain/ day
	(lb.)		
30	12	66	24
36	14	72	26
42	16	78	28
48	18	84	30
54	20	90	32
60	22	96	34

tion. (d) For every 3-lb. drop in daily milk production, decrease grain 1 lb. daily.

The protein content of the grain ration should be adjusted to balance the kinds of roughages being fed.

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PROBLEMS OF FEED EVALUATION RELATED TO FEEDING OF DAIRY COWS

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INTRODUCTION TO THE PROBLEM

It is intended that this paper concern some of the practical problems involved in the feeding of the modern, individual cow, and which demand the attention of the researcher, extension worker, and dairyman. Thus, this report will deal with a series of problems which begins with the nutritive evaluation of the ration and leads to the manger of the individual cow.

Aside from the continuing interest of the researcher and extension worker in feed evaluation generally, there has developed during the last 6 yr. a novel interest in the nutritive evaluation of forages by rapid and inexpensive, but indirect, means, with the hope ultimately that the amount and kinds of concentrates needed by the individual cow might be computed and the information relayed to the dairyman as a practical feeding recommendation. The use of indirect methods of nutritive evaluation has become known generally as forage testing. Recent, more general, use of high-speed computing devices in various centers has made this over-all scheme, including the rationing of concentrates, appear to be more feasible than it was previously.

Preliminary to considering parts of the over-all problem, it is essential to review the kinds of information needed to feed the individual cow. These consist of: (a) The amounts of nutrients needed (as set forth in quantitative guides to feeding), and (b) the quantities of nutrients provided by the feeds and rations ingested. The latter is the product of the nutritive value [e.g., total digestible nutrients (TDN)] per unit weight of feed and the amount of feed ingested. From the standpoint of feeding the individual cow, either the feeding standard or the index of nutritive value, without the other, is useless. Naturally, a shortcoming in one reduces the effectiveness of the other.

As a third consideration, certain economic conditions need to be superimposed in feeding practice upon the considerations of the relationship between the amounts of nutrients of which the cow is capable of utilizing and the amounts of nutrients provided, and the nutritive values of feeds. A wide range of intakes and of nutrive values of feeds both exists in practice and is physiologically feasible. The degree to which the amount of nutrients that the cow is capable of utilizing is satisfied by the nutritive value of the ration influences to a great extent the profits in milk production. From many feeding alternatives it is the responsibility of the researcher and extension worker to guide the dairyman in the selection of one, which currently and under local conditions, though not necessarily reducing costs, will result in maximum profits.

PROBLEMS ASSOCIATED WITH FEEDING STANDARDS

Since it is presupposed that the information obtained as a result of a forage-testing program can be used in combination with the feeding guide to ration the individual cow, a brief review of some of the bases and limitations of existing feeding standards will be made here.

Basis of feeding-standard energy allowances. The energy allowances set forth in present-day feeding standards are based chiefly on the data of Haecker (16-19). For the most part, these data were obtained with low-producing cows. For example, in 224 cow-periods comprising the Haecker experiments, the average yield of 4% FCM was only 24.4 lb. per cow per day; only 17% of the cows produced more than 30 lb.; and only one cow produced over 40 lb. of 4% FCM per day (actually, 42.6 lb.). The average intake of TDN was approximately 1.9 times the amount needed for maintenance. At these levels of milk yield, and conversely of feed intake, the TDN requirement was estimated to be 0.316 lb. per pound of 4% FCM.

On the other hand, the results of certain studies (21) of feed input–milk output indicate that cows producing about 11,000 lb. of 4% FCM per year require above the mainten-

ance allowance approximately 0.5 lb. of TDN on the average per pound of FCM produced. Although a part of this energy appears to have been used for the gain of body tissue, the size of the apparent requirement suggests that, at the level of feed intake needed to produce 11,000 lb. of 4% FCM per annum, a given quantity of TDN must have a milk-producing value per unit of weight of about 66%, of that which it would have when the same source of TDN is fed at a level slightly above that of maintenance.

Effect of level of intake on the TDN value of the ration. The TDN values recorded in tables of nutritive values and which, therefore, are used in rationing have been determined usually at levels of intake only slightly above that needed for maintenance. Though the extent of the effect of level of intake upon the digestibility of the ration is variable and is influenced by the composition of the ration, the TDN value of mixed rations comprised of concentrates and forages declines at an ever-increasing rate as the amount of ration ingested per unit of time is increased (1, 31). A hypothetical example of the plane-of-nutrition effect upon the TDN value of a given practical ration is shown in Figure 1. In working out this example, it was assumed that the TDN value of this particular hay-concentrate ration is 65%, when the level of intake is equivalent to that of maintenance, and that the composition of the ration is the same at all levels of intake or of milk output. The bases of the example in Figure 1 are the original data reported, and those of others which were reviewed, in the papers of Andersen *et al.* (1) and Reid (31), as well as the indirect evidence represented by unpublished data on the energy requirements of high-producing cows obtained by the author and his associates between 1955 and the present time. For outputs of 0 to 65 lb. of 4% FCM per day, or their corresponding feed inputs (represented by the solid segment of the line in Figure 1), the evidence for the effect of level of intake on the TDN value has a reasonable scientific basis. On the other hand, the

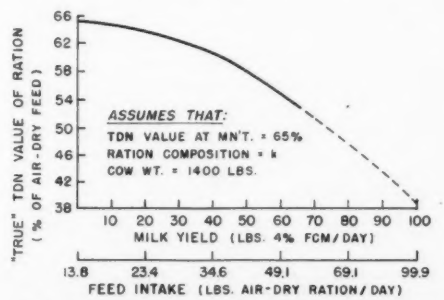

FIG. 1. Example of the effect of level of intake on the true TDN value of a ration.

effect upon the nutritive value of the ration of levels of intake needed to produce 65 to 100 lb. of 4% FCM (indicated by the broken portion of the line in Figure 1) can be documented at the present time only by very scanty data indeed.

The example employed here should not be construed to represent the relationship between the TDN value and the level of intake of all rations. It is possible that the nutritive value of some rations suffers the plane-of-nutrition effect more or less than others. For example, in the studies of Larsen and Eskedal (24), involving cows which produced 20,000 to 28,397 lb. of 4% FCM per annum, there is an indication that the level of intake had less effect upon the nutritive value of the rations fed than that which has been observed with commonly used forage-concentrate rations in America. However, an uncompromised interpretation of the Danish data is not possible, for they are complicated by the fact that the cows were allowed pasturage, the intake of which was not measured. The rations employed in the Danish work were different from the common rations used in America, chiefly in that they contained: more oil-meal feeds (a total of five) constituting a large proportion (47%) of the concentrate mixture; a high level (24%) of coconut cake in the concentrate mixture; roots constituting 18% of the total feed units (i.e., available energy) provided; and a considerably smaller quantity of cereal grains than is usually fed in the United States.

Milk-producing capacity of average cow is increasing. Mainly because of improved management, but partly because of the more general use of artificial insemination employing the semen of bulls more reliably proven to transmit high production, and because of more rigid culling of low-producing cows, the average yield per cow in the United States is increasing at a greater rate than ever before. As a consequence, the number of cows producing 15,000 lb. or more of milk per year is greater now than ever before, and almost all herds have at least a few cows which produce more than 60 lb. per day for at least a few months. For example, in New York State in 1953, 30 herds had an average annual yield per cow of 500 lb. or more of fat; in 1960, 285 herds produced at this, or a higher, level. Therefore, the problems concerned with providing sufficient energy to the high-producing cow are becoming increasingly acute and demand reappraisal of the adequacy of existing feeding standards.

Many cows would respond to greater energy inputs. Undoubtedly, many cows producing at low levels currently would yield much more milk if they were provided with more energy. This was borne out in a field study involving 442 cows in 48 herds, in which Charron (8) induced farmers to feed one-half of the cows in the test approximately 20% more of TDN than the farmers had been accustomed to feed-

ing. An increase in the annual yield of milk of 2,000 lb. or more was effected in 57% of the cows, with an increase of 4,000 lb. or more in 10% of the cows, and an average increase of 2,030 lb. was secured for all cows in the test. Another example of the increased yields resulting from greater energy inputs is represented by the demonstration conducted by Larsen and Eskedal (24) with six cows. Some of the data obtained in this demonstration are recorded in Table 1. Although a part of the increase in the response of the cows in the third year over that of the first or second year is the result of improved milking methods and health measures, as well as the normal maturing of the cows, the major part of the response is undoubtedly the result of the greatly increased input of energy. Even though these examples support the contention that greater inputs of energy would result in greater outputs of milk by many cows, it is necessary in practice to impose economic considerations on this biological fact.

Relation of body tissue gain and utilization to dietary energy allowances. It is reasonable to suspect that when milking cows are fed according to present-day standards, the body tissue gained during late lactation and the dry period caters a considerable part of the energy needed to support subsequent lactation. As a consequence, a need for dietary energy greater than the amount of TDN recommended in present-day standards may not become evident except when cows produce a large quantity of milk for extended periods of time. For example, a cow which has gained 100 lb. of body fat prior to calving could produce as much as 880 lb. of milk from the catabolism of body fat alone, when fed only enough feed to satisfy her maintenance requirements during the ensuing lactation period. Thus, if the maximum yield of potentially high-producing cows is to be realized, it would appear that either less dependence should be put on the body-tissue stores or their utilization should be more effectively distributed, and greater reliance should be put on the energy provided by the ration fed concurrently with lactation.

TABLE 1

Effect on milk yield of high inputs of feed and excellent general management [a]

Consecutive year of demonstration	4% FCM yield	Management conditions
	(lb/yr)	
1	9,325	Usual farm [b]
2	9,594	Usual farm
3	22,942	Improved [c]

[a] Data of Larsen and Eskedal (24) obtained in demonstration with same six cows during three consecutive years; average age of cows at beginning of lactation in first year was about 4.5 yr.

[b] Management by farmer-owner.

[c] Management by experiment station personnel; improved milking and sanitation practices and high-level feeding.

Further limitations of existing feeding guides. Current feeding standards are based, for the most part, on data obtained with low producers, and they assume that the energy requirement per unit of milk produced is the same at all levels of output. On the other hand, in view of the generalization represented by Figure 1, it is axiomatic that high-producing cows require in the ration considerably more of textbook TDN (i.e., TDN measured at the approximate maintenance level of intake and recorded in tables of feed values) per pound of milk than do low producers, or than that quantity recorded in existing feeding standards. The reader is alerted to the fact that although Figure 1 infers that all of the depression in the nutritive value of the ration (since it is expressed in terms of TDN) effected by increasing planes of nutrition is the result of reduced digestibility, some of the reduction in the milk-producing value of the ration is the result of a progressively less efficient utilization of absorbed matter as the level of intake is increased. However, it appears that the latter effect of the level of intake is responsible for only a small proportion of the over-all reduction in the milk-producing value of the ration. These two general conditions would account for the depression in the milk-producing value of ration when a state of energy equilibrium exists in the cow's body. Needless to state, this is seldom the situation in practice. Increasing levels of inputs usually result in increasing rates of body gain. As a consequence, body gain represents a further reason for the ever-decreasing milk-producing value of the ration as the intake increases (6, 31, 32). Nevertheless, from the standpoint of rationing the individual cow, the over-all effect is the same; more textbook TDN is needed to produce a unit of milk at a high level, than at a low level, of output.

A tentative energy standard for milk production. In view of the apparent limitations of present-day energy standards for milk production cited above, and the apparently more acute needs of the modern cow, an attempt has been made in Figure 2 to set forth a tentative feeding standard for milk production by cows which are neither gaining nor losing energy from the body. This standard is predicted on the basis that the true TDN value of rations made up of increasingly greater proportions of concentrates declines as the level of intake increases, and the maintenance requirement of the 1,000-lb. cow is 7.0 lb. TDN per day (14, 32, 40, 41) and varies for cows of other sizes according to body weight $^{0.73}_{\text{lb.}}$.

The graduated allowances represented by Figure 2 are a hybrid of the theoretical principles governing the utilization of energy by the cow (31, 32) and the TDN requirements

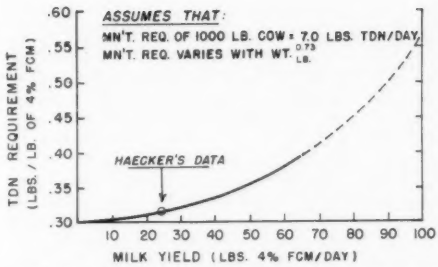

FIG. 2. Tentative feeding standard for milk production by cows not gaining or losing body tissue.

determined by Haecker (16-19) with low-producing cows and those of the author and his coworkers estimated from data obtained with medium- to high-producing cows. The basis of the requirement for cows yielding up to 65 lb. of 4% FCM per day (as indicated by the solid line in Figure 2) is more reliable than that of the requirement for cows producing above this level (represented by the broken line). It is emphasized that the proposed standard is tentative, subject to change with further study, and intended to serve as a guide only until more adequate data become available.

Influence of body tissue gain and utilization on the dietary requirement for energy. Although Figure 2 is intended to represent the TDN needs of lactation by cows in energy equilibrium, lactation and its incidental processes are dynamic in nature and, therefore, influence the dietary requirement for TDN with respect to time during the lactation period and the distribution of TDN inputs during certain periods of time. Figure 3 illustrates some of the processes incidental to lactation and particularly the degree to which body tissue can be gained and lost at various stages of the lactation cycle.

The data from which the diagram was prepared were obtained in long-time feeding trials at Cornell, in which body weight measurements were made just before and after parturition and at weekly intervals during other times. During the dry period, the level of feeding was

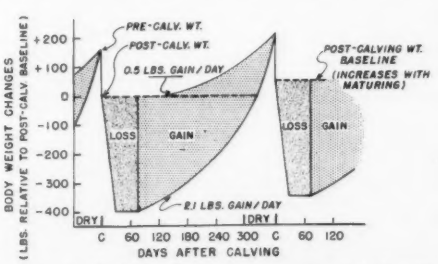

FIG. 3. Body weight changes associated with pregnancy and lactation in Holstein cows.

sufficient to effect a state of body condition that satisfied a subjective visual standard. During the first seven- to ten-day period after calving, 10 lb. of concentrates and 36 to 40 lb. of corn silage were fed per day and hay was provided ad libitum. When the cows were on pasture, similar quantities of concentrates were provided, but without hay or silage. Thereafter, the level of concentrates was increased gradually to meet the feeding-standard energy allowance. As a consequence, the feed input followed, rather than led, the milk output.

These data show that the loss of body weight (presumably body tissue) after calving usually reaches a maximum within 30 to 75 days postpartum. For most cows the minimum weight was recorded within 60 days post-partum. Naturally, the extent of body tissue losses depends greatly upon the amount of feed ingested during early lactation and the amount of milk produced, as well as the amount of tissue available. It was observed that most cows fed according to the plan outlined above lost from 100 to 200 lb., with an average loss of about 150 lb. However, some of the cows lost as much as 400 lb. Body-tissue losses of similar magnitude are suspected to occur in practice. It is axiomatic that the amount of body tissue lost during early lactation determines the amount that must be gained during later lactation and the dry period. Since the use of dietary energy to gain body tissue and its subsequent conversion to milk is much less efficient energetically than the direct conversion of feed energy to milk (31), the amount of body tissue anabolized and subsequently catabolized should be kept at a minimum.

Other practical implications of the events diagrammed in Figure 3 are that: (a) Body tissue as a source of energy for milk production should be depended upon to a minimum degree, (b) the necessary gain of body tissue should occur chiefly during the dry period, when the feed intake is lower and, therefore, when the efficiency of feed utilization is greater than it is during lactation, and (c) greater attention needs to be given to the distribution of the energy inputs during the lactation cycle.

The alternate gaining and losing of body tissue exert significant influences on the utilization of dietary energy by, and, therefore, on the practical rationing of, the individual cow. Some of these effects are: (a) The use of dietary energy for body gain contributes to reduced outputs of milk per unit of feed input. (b) The energy resulting from the catabolism of body tissue supplements the energy of the diet for high-producing, and underfed, cows. Thus, some degree of body-tissue utilization is an absolute essential if the maximum yield is to be realized by those cows whose milk-producing potential exceeds the limits allowed by their appetite or stomach capacity, and the nutritive quality of the ration provided. (c) As a consequence, the anabolic-catabolic processes have a

marked effect upon the amount of dietary energy needed for lactation.

An attempt has been made in Figure 4 to diagram the approximate influence of the utilization of various quantities of body tissue during the first 75 days of lactation, upon the dietary energy requirements for the production of various quantities of milk during the same period. The bases of these data are the requirements shown in Figure 2, upon which were superimposed the calorie gains of mature Holstein steers (39) gaining body tissue at the rates required by cows during 290 days, in order to have available for catabolism during the first 75 days post-partum 0 to 400 lb. of

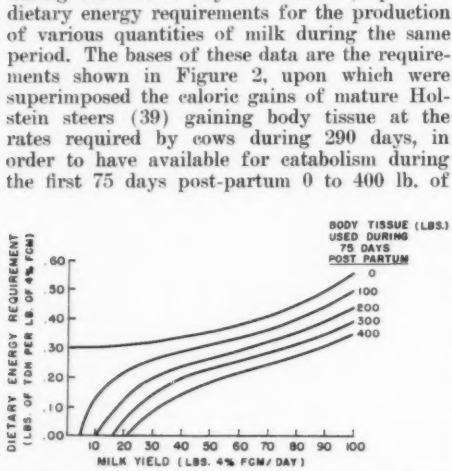

FIG. 4. Effect of amount of body tissue used during first 75 days post-partum on the TDN requirement for milk production during the same period of time.

body tissue. Although Figure 4 represents a feeding standard for milk production during the first 75 days of lactation, the level of production combined with the amount of feed ingested determines the extent of tissue utilization. But, in practice the amount of tissue available for use and the amount being used at a given time are difficult to determine. It is essential that further studies of the energy requirements for milk production by the cow take into account the incidental energy transactions of the cow's body.

High milk yield per cow is an economic necessity. The data which constitute the basis of Figures 3 and 4 indicate that in practice it is important to provide the amount of feed the cow needs concurrently to produce the quantity of milk of which she is capable. Naturally, the superimposition of certain economic conditions will modify the degree to which the dietary needs should be fulfilled in practice. However, it is important to recognize that the mere cost of feed is by no means the only form, or necessarily the larger proportion, of monetary outlay. For example, the efficiency with which a cow producing 80 lb. of milk per day utilizes the energy ingested above the maintenance level is considerably less (therefore, she requires more feed per pound of milk produced) than that of two barn-mates, each of which is producing 40 lb. per day, or a total of 80 lb. In other words, the feed cost above maintenance for the same total milk yield favors the two lower producers rather than the one high producer. On the other hand, the body maintenance cost, the space and equipment investments, and the labor requirements are considerably lower per unit of milk produced by the high-producing cow than they are for her two lower-producing barn-mates. Thus, the economic efficiency of the high-producing cow is usually greater than that of the low-producing cow, even though the energetic efficiency of lactation may be somewhat greater for the low producer.

Maldistribution of energy inputs during the lactation cycle. Another point of significance to the feeding of the individual cow suggested by Figures 3 and 4 is the consequence of the maldistribution of energy during the lactation cycle. There is evidence that the minimum feeding standards are not being used generally or effectively in practice. Although many cows do not get enough feed at any time, some cows receive enough total feed per annum, but the feed is distributed ineffectively relative to time and need. A defect commonly encountered by extension workers is that in which too little feed is provided early in lactation and too much late in lactation. This feeding defect is demonstrated by the diagrams in Figures 5 and 6, based upon data obtained for two DHIA herds in New York State and studied by Warner (46).

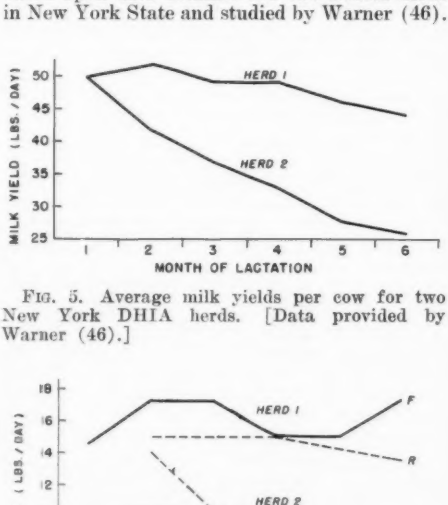

FIG. 5. Average milk yields per cow for two New York DHIA herds. [Data provided by Warner (46).]

FIG. 6. Average amounts of concentrates fed and recommended per cow in two New York DHIA herds. [Data provided by Warner (46).]

In Figure 5, it will be noted that the milk yields of the two herds were the same in the first month of lactation, but that Herd 1 was more persistent and produced more milk in all subsequent months than did Herd 2. At least a partial explanation for the difference in responses of the two herds is provided in Figure 6.

The amount of concentrates fed to Herd 1 during early lactation provided enough energy to exceed the recommendations of present-day energy standards. On the same basis, Herd 2 was greatly underfed during early lactation, and overfed during the 4th, 5th, and 6th months of lactation. Despite the fact that the cows in Herd 2 received a considerably larger total amount of concentrates during the 6-mo. period than was recommended, the milk yield declined to a low level quite rapidly, with the resulting low total yield. The superior yield and the persistency of output of Herd 1 would appear to be the result partly of the higher energy input and partly of the more satisfactory distribution of the energy input.

It is important that cows be provided enough energy to produce as much milk as possible as soon after calving as possible. This is more because of the total lactation-period response than because of the response during the first few months' lactation. The well-documented finding (13, 23, 27, 28, 36, 37, 38, 43, 44), that the milk yield during monthly segments of the lactation period (even very early in lactation) reflects the total lactation-period yield, suggests this conclusion. Furthermore, the response in milk yield to energy inputs during the dry period and early lactation has been found to be cumulative over the lactation period (5-7, 12). The size of the milk-production response to energy input during the dry period appears to be influenced by the condition of the cow upon entering the dry period and the level of intake provided after calving. Milk output, at least during early lactation, has been correlated with the input of energy during the dry period (5, 7, 12, 25). Continued feeding of high levels of energy after calving has extended the increased output of milk over the entire lactation period (5-7). The response to increased intakes during the dry period either has not occurred at all in some studies (15, 30, 35) or has diminished as lactation proceeded in others (12, 25), when the control, and high-level, cows were fed at the same rate after calving. Although the relationship of early-lactation yield to total lactation-period yield, and the cumulative effect on production of prior nutritional treatment are well established, their physiological bases have not been unraveled.

Summary of feeding-standard limitations and common defects in practical feeding. In summarization of the previous discussion, it is concluded that some of the major problems concerned with the practical feeding of the individual cow are associated with the inadequacies either of the present-day feeding guides or of their application. These are: (a) Existing feeding standards become progressively less adequate as the milk output increases per unit of time. (b) The feeding standards do not take into account the storage and use of body tissue with relation to the production of milk; as a consequence, too much reliance is put upon body tissue catabolism to provide the energy lacking in the diet. (c) The lack of acceptance, or the ineffective use, of even the minimum energy recommendations set forth in the feeding standards frequently results in the provision of too little feed and in the maldistribution of feed input during the lactation period. (d) The usual application of the feeding standard allows past milk yield to dictate future feed inputs, with the consequent accentuation of the maldistribution of feed input.

PROBLEMS CONCERNING THE NUTRITIVE EVALUATION AND VALUE OF FEEDS AND RATIONS

Requirements of a forage-evaluation method to be used as a tool in rationing the individual cow. Next, some of the problems associated with feed evaluation and values will be considered in relation to the defects in present-day feeding practices discussed earlier in this report. At the onset, and in view of (a) the inadequacies of the present feeding guides, (b) the ineffective distribution of the feed inputs, and (c) the uncertainties of body-tissue usage, the question is asked: "Can feed (or forage) evaluation schemes short of direct measurements be expected to provide information which will lead to definite improvements in the milk yield per cow and in the economics of milk production?" To develop an answer to this question, it is necessary to consider the nature of the information being obtained in present-day feed-evaluation programs.

From the standpoint of feeding the individual cow, the problems of feed evaluation are chiefly those concerned with forage evaluation. This is because the commonly used mixed concentrates have a relatively constant nutritive value per unit of weight and the quantities of concentrates fed to the dairy cow are usually measured. (Nevertheless, the relation to the nutritive value of rations of various proportions of concentrates and forages, and the effect of the level of intake on the nutritive value per pound of such mixtures, need much further study.) As a consequence, the ultimate purpose hoped to be served by a forage-evaluation program is that of determining the amount of concentrates needed in the ration with a particular forage(s) to satisfy the needs of the individual cow, as indicated by the feeding standard. Reasonably accurate rationing of concentrates is possible only when both (a) the nutritive value (e.g., TDN) per unit of weight of forages and (b) the amount of forages ingested are known.

But, even with complete information on the nutrient concentration and intake of forages, the accuracy with which concentrates can be rationed is limited to the degree of adequacy of the feeding guide followed.

Kinds of information provided by existing forage-evaluation schemes. The forage-evaluation schemes in vogue currently provide for the prediction of the nutritive value from the concentration of certain chemical substances (2, 3, 20, 22, 29), certain criteria of in vitro fermentation by ruminal bacteria (4, 9-11, 20, 26, 34, 42), and the date when forage is harvested (33). (Only a few recent references are cited here; the reader can obtain a more extensive bibliography in these papers.) In general, the forage-testing procedures provide an estimate of the TDN value (or a related criterion) per unit weight of forage. Since the prediction equations employed in these methods were derived using the conventional digestion trial as the standard, the TDN values so estimated are representative of those which exist at about the maintenance level of intake when forage constitutes the entire ration. (Some forage-evaluation programs provide an estimate of the digestible protein value. But, since protein, as compared with energy, is relatively unimportant from both the quantitative-nutritional and economic viewpoints, provided that a certain minimum requirement is satisfied, no attention will be given to this nutrient here.) None of the forage-evaluation methods, except the Macdonald College method (9-11, 26), provides for an estimate of intake or of over-all nutritive value, and this procedure has not been examined with cattle.

Range in TDN value and intake of forages in practice. In practice, the TDN value of perennial forages ranges from 50 to 68% on the dry basis and the dry matter intake ranges from 1 to 3.5 lb. per 100 lb. of body weight per day. A change from the lowest to highest quality of forage used on dairy farms would result in an improvement of 36% in TDN value and of 250% in the dry matter intake. These two criteria of forage quality are to a considerable degree positively correlated, particularly for first-growth forages. Thus, as forage quality is improved, the dry matter intake increases on the average at a rate approximately seven times as great as that of the TDN value per pound. From this, it is rationalized that the increase in total energy intake from one quality of forage to another is approximately 90% attributable to the increased intake and 10% attributable to the improvement in TDN concentration. On the same basis, it is expected that a method which would provide an estimate of intake with a precision equal to the estimation of the TDN value by forage-evaluation methods would result in a great error in the prediction of the intake of forage energy.

Some practical information not provided by present forage-testing schemes. The following practical conditions are not accounted for by forage-testing schemes in their present form and, therefore, from the standpoint of feeding the individual cow, they correspondingly limit the value of the information obtained:

(a) Amount of forage eaten.

(b) Effect of the amount of concentrates fed on the amount of forage eaten.

(c) Degree of selective feeding (i.e., proportion of leaves and stems ingested). The extent of this act has an appreciable effect upon the energy concentration of the forage ingested. For example, a refusal of stems, and their replacement with leaves, equivalent to 10% of the dry matter offered, could increase the TDN concentration by as much as 5 to 12%.

(d) Effect of physical form (viz., long, chopped, finely ground, dry and wet) on milk-producing value of ration. At the present time, physical form does not represent a serious practical problem, because most forage is fed to dairy cattle in long or chopped form. Increased use of finely ground forage may make physical form more important in the future, because particle size influences the intake, digestibility, and the end products of digestion which are absorbed.

(e) Milk-production efficiency of the absorbed mixture of digested end products of varying chemical nature.

(f) Degree of wastage of absorbed matter (or conversely, efficiency with which absorbed matter is utilized).

(g) Effect of the level of intake on the nutritive value of the ration. However, the importance of this limitation is diminished to the degree that the feeding guide employed accounts for the plane-of-nutrition effect.

(h) Frequency of feeding. Although frequency of feeding has a significant effect on the response of growing cattle, it does not affect the yield of milking cows, except when it results in an increased intake of feed.

Relation of information obtained by forage-testing to the rationing of the individual cow. Since certain intangible benefits such as the possible awakening or renewal of farmer interest in forage quality might be attributable to forage testing, it is difficult to evaluate the fruits of such testing in quantitative terms. On the other hand, the ultimate aim of forage-testing programs is the rationing of concentrates to the individual cow. As a consequence, a quantitative perspective of the contribution of forage testing to the total effectiveness with which concentrates can be rationed will be

clarified in the following statements. Let us assume that the rationing of concentrates to the individual cow is 100% effective, when accurate information on the energy intake in forage and the most adequate recommendations of energy feeding standards each represents 50% of the possible total effectiveness. As rationalized earlier, increments in the total energy intake in forage as quality is changed are attributable to the dry matter intake and TDN concentration to the degree of about 90 and 10%, respectively. On this basis, knowledge of a perfectly precise TDN value for the forage would contribute only 5% of the total effectiveness with which concentrates can be rationed. (As will be pointed out later, even if this contribution were 25%, the final conclusion would be the same.) There is no doubt that forage testing represents somewhat of a refinement over the use of existing tables of TDN values of forages, or the use of a median TDN value (e.g., 59% on dry basis). For example, assuming the median TDN value of 59% to represent the range of TDN values (viz., 50 to 68%) found in practice could result in a maximum overestimation by 18% and a maximum underestimation by 15%. These figures represent the degree of potential refinement that might be effected in the estimation of the TDN value by forage testing. However, even if it is assumed that a perfect estimate of the TDN concentration is provided by forage testing, rationing concentrates on the basis of forage testing is less than 1% more effective over-all than computing the amount of concentrates on the basis that the forage contains 59% TDN. Stated in other words, this means that concentrates can be rationed to the individual cow 99% as effectively without forage testing as with it. Should there be as much as a five-fold error in this rationale, the conclusion would be very little different; concentrates could be rationed 95% as effectively without, as with, forage testing. Therefore, even if a forage-testing scheme provided a perfectly accurate estimate of the TDN value per pound, it would contribute very little information having intrinsic value to the rationing of the individual cow, entirely because, in their present form, the forage-testing methods do not appraise the intake.

There is reason to believe that refinements in estimating the intake of forage, the use in practice of more adequate and precise feeding guides, and a more effective distribution of feed during the lactation cycle would improve the effectiveness with which concentrates are rationed by as much as 40 to 50%.

Thus, it is concluded that no forage-testing program can be considered to be adequate, or even very helpful, unless both the intake and the nutritive value per unit of weight can be predicted with sufficient accuracy to ration the individual cow under practical conditions. (None of the forage-testing schemes in their present form or mode of application provides estimates of both criteria of nutritive value.) But, even if a satisfactory procedure existed, the successful execution of such a program extended ultimately to rationing the individual cow requires, in addition to information on the total energy ingested as forage, the application of a feeding standard that (a) both anticipates and satisfies the needs of current production, as well as (b) provides for the effective distribution of the feed input during the dry period and the lactation period.

The main thesis of this report is that, in order to ration concentrates to the individual cow (the ultimate aim of forage-testing programs), information on both the intake and nutritive value per unit weight of forage is needed, as well as an adequate feeding standard. Shortcomings in any of these naturally limit the accuracy with which concentrates can be rationed.

There are several serious weaknesses in present-day feeding practices associated with the inadequacies and ineffective applications of existing feeding standards. It is submitted that, if these were remedied, infinitely more would be done to improve the milk output per cow and the economics of production than can be accomplished by forage-testing schemes, particularly in their present form. Fruition of this statement requires that acceptance of certain improved practices by dairymen be gained and maintained.

Gaining farmer acceptance and maintaining farmer usage of worthwhile practices. Almost all of the improvement in milk production and monetary income attributed to forage testing by the popular press (45, 47) is really the result of feeding more concentrates and distributing the input of feed more effectively. Naturally, both of these can be done without forage testing. Nevertheless, attributing the benefits obtained to forage testing is misleading and represents an ungenuine demonstration. This is an example of an extension-teaching method in which the tools of dramatics, rather than the subject matter being dramatized, are emphasized. It would seem that if farmer acceptance of any worthwhile program is to be gained and maintained, the extension worker must teach the farmer principles, rather than lead him to some stop-gap end with transient thumb rules, gimmicks, and temporary crutches. Principles have the peculiar qualities of stable longevity and general application, which are not shared by other foundations of teachings.

Some of the forage-testing schemes which are not based upon, or do not teach, a principle can be categorized as gimmicks. It is sometimes stated that forage testing makes the dairyman conscious of forage quality; this is an advantage, if true. But, the important question is: "For what practical purpose can the dairyman use the information gained by forage testing?" Because he is being made in-

creasingly aware of forage quality, sooner or later he will learn that the answer to this question is: "Essentially no purpose."

A SIMPLE FEEDING PLAN

As the dairyman is taught principles more and more, and becomes more conversant with them, he will learn why certain principles are true and what monetary value their application has. He will learn, for example, that a high milk yield per cow is an economic necessity, that the nutritive value of early-cut forage is higher than that of late-cut forage, and that the feeding-standard energy allowance is just a minimum guide line to which he can add under certain situations to exact a more profitable yield from certain cows. Dairymen can be taught certain principles which will provide them with the means to improve upon the minimum guide lines set forth in general rules of thumb. It seems far less important that the dairyman applies the rule book to the last pound than he applies principles which guide his business towards general improvement and greater profit.

For example, the dairyman who is aware of feeding principles can do a more effective job by following an informal plan, such as that outlined below, than he can by using existing feeding standards and tables of average feed values in cook-book fashion. This plan is not new, but it represents a synthesis of feeding principles. It is proposed here mainly because of the shortcomings of existing feeding standards and particularly in view of the lack of, or ineffective, use of the feeding standards in practice. This plan consists of: (a) The ad libitum feeding of the best quality forages available. (b) Flexible energy allowances during the last two months of the dry period which, in some instances and depending on the condition of the cow, would be provided by a ration ranging from all forage to forage plus 8 to 10 lb. of concentrates during the first 4 to 6 wk., and a gradually increasing level of concentrates thereafter, reaching a level of 15 to 18 lb. by the time of calving. This scheme would appear to have two benefits: the storage of body tissue and, perhaps more important, accustoming the cow to consume large quantities of feed. (c) After calving, increasing the level of concentrates as rapidly as possible to either maximum appetite or maximum milk yield, whichever is attained first. The cow should decide her level of intake at this time; the dairyman must learn by trial the rate at which the individual cow's intake can be increased. (d) Thereafter, the level of concentrates should be reduced to the lowest level which does not reduce the milk yield. In this way feed intake tends to lead the milk output, rather than the reverse. A change in the amount of feed ingested is usually reflected in the milk yield within 30 hr. Thus, with close attention, prolonged periods of underfeeding or overfeeding can be avoided.

This plan for feeding the individual cow is simple and practical to execute, does not require the use of feeding standards and tables of feed values (therefore, it obviates the need for forage testing, and an estimate of forage intake), and provides for an effective distribution of feed during the lactation cycle. One of its major features is that of preparing the cow for a large feed intake very soon after calving. In this way, advantage can be taken of a high yield early in lactation, with the consequent cumulative effects upon the milk yield during the entire lactation period (5-7, 12) to exact a large total-lactation yield. It has been demonstrated that heavy feeding during the dry period is without effect upon the severity of udder edema, or on the incidence of milk fever or ketosis (15, 30, 35).

The plan suggested has the further advantages that the provision of feed leads, rather than follows, the output of milk, and it avoids lags in feed input relative to milk output which exist in the usual application of the feeding standard and in programs involving the series of events of forage testing, the central computation of data concerned with production and feed allowances, and the relaying of concentrate-feeding recommendations to the dairyman. Doubtless, the latter programs have the advantage of attracting interest.

Such a feeding plan, however, puts more daily management pressure on the dairyman and requires that each cow be given individual attention. Although it can be argued on some bases that, with increasing size of herds, less attention can be afforded the individual cow, the degree of individual attention that is most profitable must be considered in relation to other economic alternatives. There is no doubt that the daily rationing of feed is one of several management operations that is more profitably done on the individual cow, rather than on the less personal, herd, basis.

SUMMARY

To ration concentrates effectively to the individual cow, the dairyman needs to know (a) how much TDN the cow needs and how much can be fed profitably, (b) the TDN value of the forage to be fed, and (c) how much of the particular forage will be ingested. Thus, effective rationing requires a satisfactory feeding standard and information on the over-all nutritive value of the particular forage(s) to be fed. A limitation in either the feeding guide employed or the appraisal of the nutritive value of the forage naturally is reflected accordingly in the amount of concentrates fed.

Recent attempts have been made to develop rapid and inexpensive, but indirect, methods for the assessment of the nutritive value of forage in programs which have become known

as forage testing. The ultimate aim in the use of a forage-testing program is that of determining the kind and amount of concentrates that should be included in the ration of the individual cow as dictated by the feeding standard. Although forage-testing schemes in their present form and manner of application provide an estimate of the TDN value per unit weight of forage, they do not provide an estimate of the more influential determinant of the total energy intake, the expected forage intake. As a consequence of this limitation, and others which have been detailed in the text, it is concluded that forage-testing schemes contribute very little information of genuine value to the daily rationing and the improvement in milk yield of the individual cow. This is particularly true when the shortcomings of forage testing are considered in relation to the limitations of existing feeding standards and certain commonly encountered defects in practical feeding.

Existing energy-feeding standards are both inadequate and ineffectively used in dairy farming practice. Some of the apparent shortcomings of present-day energy standards are indicated by the following conditions: (a) The data on which the standards are based were obtained with low-producing cows, (b) though the degree of effect varies from ration to ration, increasing inputs of feed result in an ever-decreasing nutritive value (e.g., TDN) per unit of feed ingested and an increasing rate of body-tissue gain, (c) as a consequence, increasing inputs of energy above the existing feeding-standard allowances result in increasing outputs of milk, though at an ever-decreasing rate, (d) the milk-yielding potential of the dairy cow is increasing gradually and many more very high producers exist now than ever before; thus, existing energy standards are much too low for many cows now and are becoming progressively less adequate with passing time, even for the national average herd, (e) current feeding standards take very little, or no, account of the effects on production of dry-period feeding and the consequent storage, and subsequent utilization, of body tissue as a source of milk energy, (f) the TDN values recorded in tables of feed values and used in conjunction with feeding standards for the purpose of rationing have been determined usually at about the maintenance level of intake, and (g) in view of this [(f) above], and the depressing effect of increasing planes of nutrition on the milk-producing values of the ration [(b) above], it is obvious that the dietary energy requirement per unit of milk produced increases with increasing level of feed intake or, conversely, with increasing output of milk, per unit of time.

In view of the limitations of existing feeding standards, a tentative energy standard is presented here in which the TDN allowances are graduated in terms of milk output per day. This attempt to develop a more versatile feeding guide, which accounts for the level-of-intake effect, is based in part upon theoretical considerations and in part on directly determined requirements. In this standard the following amounts (lb.) of TDN above the maintenance allowance are recommended for the production of 1 lb. of 4% FCM, when the ranges in daily milk yield (lb.) are as follows: 0 to 10, 0.30; 11 to 20, 0.31; 21 to 30, 0.32; 31 to 40, 0.33; 41 to 50, 0.35; 51 to 60, 0.37; 61 to 70, 0.40; 71 to 80, 0.43; 81 to 90, 0.47; and 91 to 100, 0.53.

Body tissue gain or utilization modifies the dietary requirement for energy. For example, the utilization of 100 lb. of body tissue could provide sufficient energy to support the production of approximately 400 lb. of 4% FCM. As a consequence, the utilization of body tissue, which (when it occurs) usually occurs during the first 75 days of lactation, would reduce the dietary requirement correspondingly during this period of time.

The lack of, or ineffective, use of existing feeding standards contributes to other defects in feeding practice. Some cows receive insufficient amounts of energy at all times. Other cows receive a sufficient total amount of feed per year, but it is distributed ineffectively. A common expression of this defect is that of providing too little feed early in lactation and too much feed late in the lactation period.

Associated with this is the failure to make effective use of the tissue gained during the dry period. Feeding plans followed in practice during early lactation demand that too large a proportion of the energy needed for milk production be provided by the catabolism of body tissue. Some degree of tissue utilization is inevitable for those cows whose milk-producing potential exceeds the limits of their appetite or stomach capacity. Also, this process is a necessity if the high-producing cow is to realize her maximum yield. Nevertheless, the alternate gaining of body tissue and its subsequent use to support lactation is inefficient energetically and should be restricted to a minimum.

The finding by dairy-cattle geneticists that short-period lactation records reflect the total lactation-period yield suggests that feed inputs during the dry period and early lactation have a cumulative effect on the yield of milk throughout the lactation period. As a consequence, it is essential that the input of energy be so distributed that the output of milk reach the maximum level as soon as possible after calving, in order to attain the maximum yield for the total lactation period.

A related time-distribution of input defect inherent in the application of all feeding standards needs to be remedied. This one is concerned with the fact that milk output dictates what the feed input should be, with a considerable time lag.

In view of the shortcomings of forage-testing

schemes and existing feeding standards, and the common defects in practical feeding, a simple feeding plan is proposed as a remedy. The main features of this plan are (a) the ad libitum feeding of high-quality forage, (b) accustoming the cow to a large intake of concentrates gradually, beginning a few weeks before the end of gestation, (c) after calving, allowing the cow to set her own pace on her intake of concentrates under the careful scrutiny of the dairyman and until she attains her maximum appetite or milk yield, (d) after this stage, determining by astute experimentation the lowest level of concentrates which is without effect upon the milk yield. This plan obviates the need for energy-feeding standards and tables of feed value and provides for the effective distribution of feed during the lactation cycle. However, it requires the measurement of the milk output and close attention to the individual cow.

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USING FEED INFORMATION ON THE FARM

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Over the past three decades research workers, as profoundly typified by Dr. Huffman and Dr. Reid, who have presented papers at this Symposium, have researched and reported many significant findings in the subject matter area of dairy cattle nutrition. When we view the present U. S. average yearly per cow production of 7,004 lb. in the light of nutritional information available and the stepped-up genetic production potential, as brought about to a large degree by our artificial breeding associations, I believe we can only conclude that a supreme challenge is at hand for all of us vitally concerned with stimulating dairymen to effectively utilize the dairy cattle feeding information now at hand.

Agricultural extension workers have carried the primary responsibility for this monumental task of making dairymen aware of, encouraging them to try and adopt, new practices on their farms. Today, I am convinced that extension dairy specialists have a large group of men giving them a significant assist—these men are employed by feed manufacturers throughout the United States. This force is composed of the dairy specialists in feed industry research and sales departments, and the larger group of sales-service representatives who are daily calling directly on dairymen. These men and extension dairy specialists have a common primary objective: assisting dairymen to increase net profits from their dairy enterprises. In the few minutes at my disposal today, I want to apprise you of what one feed manufacturing concern is doing in this area of using feed information on the farm.

The Beacon Milling Company has had a specific dairy feeding program for over 20 yr. Somewhere along the line a vital link or two

was missing, because a relatively small number of Beacon Advisors were selling program along with the grain. As a result, a limited number of feeders were using our program.

In late 1956, we began a careful study of this matter and the result was what we call Dairy Feed Programming. It was clear to us that it was necessary to develop a grain-feeding program based more specifically on nutrient requirements. Time and again, it was being noted that grain-to-milk ratios were underfeeding the high-producing cows and overfeeding the low producers. It was also evident that increased attention had to be focused on evaluating the quality of the roughage available for feeding on a given farm.

Grain feeding tables were developed utilizing the Morrison Feeding Standard as the reference for TDN requirements. Roughage quality was broken down into five categories of hay equivalent intake per 100 lb. of body weight, namely: 2.75, 2.50, 2.25, 1.8, and 1.5. Total digestible nutrient values were assigned to the five quality levels of hay, silage, and pasture. Percentage fat was accounted for in three ranges, namely: 3.0-3.7, 3.8-4.4, and 4.5-6.0.

Special attention is placed on dry and fresh cow feeding. A special fresh cow–feeding guide is available for cows capable of moving above 65 lb. of 4% milk. It is designed to bring the higher-potential cows onto full feed within 2 wk. following freshening. In practice, we find that this special fresh-cow schedule has to be used with discretion. When cows commence consuming above 20 lb. of grain daily, the importance of the feeder increases markedly.

Beacon Dairy Feed Programming is based on the following procedures:

A. A careful evaluation of the quality and quantity of roughages available for feeding. A combination of laboratory analyses, cutting dates, and amounts being fed is used in arriving at the probable hay equivalent intake per 100 lb. of body weight. The hay equivalent intake level forms the basis for determining the level of grain feeding for any given cow.

B. Determining the daily grain intake for each cow monthly, based on milk production, body weight, and condition, and milk fat test. The grain feeding table is used as a guide.

C. Selecting the crude protein level in the grain ration to assure adequate protein intake. The roughage crude protein content as obtained in the laboratory is used to help make this decision.

D. Monthly inspection by the Beacon Advisor of roughage being fed, amounts of roughage being consumed, body condition, and production level. New grain feeding charts are made up and feeding and management are discussed.

Following the development of the grain feeding tables and special fresh cow–feeding guide, these tools were immediately placed into use with our research herd in mid-1957. Eleven cows in the purebred Holstein research herd fell in the age bracket of 5 to 8 yr. as the new grain-feeding program was begun. Table 1 summarizes their 1958 performance, as compared with the 1957 production made while on the old program.

TABLE 1

Comparison of production performance of Beacon Dairy Research herd cows in two consecutive years [a]

	(lb. milk)	Average age start of records
1958 New program	18,347	6-4
1957 Old program	16,885	5-3
Average per cow increase	1,462	

[a] Eleven cows-2×-305 days-M.E.

Though we are unable to determine just what portion of this increase in production was due to the new program, we are confident it made a significant contribution. Over-all management and personnel were unchanged. A suppplemental heat batch hay dryer has been in use at the research farm since 1953, and tends to smooth out peaks and troughs in our hay quality.

With milk valued at $4.40 per hundredweight, each cow increased her gross lactational return $64.32. On the new program, these cows received higher levels of grain early in lactation and considerably less after midlactation. The net average increase in grain intake was approximately 360 lb. of grain. With grain at $4.20 per hundredweight, each cow in 1958 returned $49.20 more above feed cost. Grain intake per cow could have been profitably increased close to 1,500 lb.

An integral part of Dairy Feed Programming is our roughage analysis service. Beacon Advisors are equipped with Penn State Forage Samplers. Forage samples, along with completed information forms giving the following information, are forwarded to our central laboratory at Cayuga:

A. Plant types
B. Cutting (1-2-3)
C. Date of cutting
D. Curing method
E. Tons available
F. Pounds fed daily
G. Stage of maturity for corn silage

Once the laboratory data are available to our department, we determine the probable hay equivalent intake per 100 lb. of body weight characteristic of the herd in question. We rely heavily on cutting date information and silage moisture in arriving at the hay equivalent figure. The hay crude protein level assists us in making recommendations as to grain crude protein level. From the time the samples are taken on the farm, until our recommendations along with the laboratory results are in the hands of a Beacon Advisor, about ten days have elapsed. The Advisor and the dairyman then go over the results together and the herd is programmed. The Advisor checks back to adjust grain levels, and counsels with the feeder at least monthly.

We concentrate on working with dairymen enrolled in some form of testing program. If a given feeder is not testing, he is urged to do so. If this fails, he is encouraged to obtain milk weights for two consecutive milkings just prior to the Advisor's visit. If the feeder can not be encouraged to even go that far, we have many cases where Advisors weighed the milk to get the dairyman interested to the point where he would carry on. Once they can see progress, the increased interest in their cattle exhibited by a good number of feeders has been highly interesting and rewarding.

What has occurred as Beacon Advisors have helped dairymen program-feed? To help provide an answer to the question of how feed-programming is performing on the farm, a summary was recently made of the performance of DHIA herds that were willing to make end-of-year summaries available. Results of this study are given in Table 2.

I am certain we can all agree that to be effective in getting a dairyman to use improved feeding practices, we must first be successful in arousing his interest to the point where he will carefully consider the idea we are trying to sell. In this challenging area, those of us in industry must have a reasonably good batting average or we don't even get to warm the bench. Fortunately, in recent years, while competition has become increasingly keen, our batting average has been at an all-time high. We attribute much of this success to feed-programming and our ability to move dairymen from the awareness stage to the adoption stage in a relatively short span of time. Of equal importance, we have been able to come up with long intervals of feeder adoption.

As dairy operations become larger, and the operators become better businessmen, we have shifted our selling methodology. We are concentrating on appointment selling. If we have the undivided attention of the feeder at his kitchen table, we can effectively go over the reasons for feed-programming, its development, the details of the program, and then go over what it is doing for fellow-dairymen.

TABLE 2

Performance pattern of programmed herds [a]

Three consecutive years on program
(Average increases for 250 cows in seven of the 36 herds)

	Milk	Fat	Income over feed cost [a]
	(lb.)		
First year	488	20.0	$ 5.56
Second year	807	26.5	31.14
Third year	1,004	40.1	20.01
Total 3-yr. increase	2,299	86.6	$56.71

Two consecutive years on program
(Average increases for 773 cows in 20 of the 36 herds)

	Milk	Fat	Income over feed cost [a]
	(lb.)		
First year	610	22.7	$16.54
Second year	706	33.1	27.60
	1,316	55.8	$44.14

First year on program
(Average increases for 1,476 cows in 36 herds)

	Milk	Fat	Income over feed cost [a]
	(lb.)		
One-year increase	724	26.9	$16.47

[a] Same price per hundredweight of milk used for year prior to programming and for each year on program.

In some cases, the prospective feeder is taken to talk with a fellow-dairyman who is programming and has had an opportunity to observe its benefits. This much time can not be given to every prospect, but it can be justified with dairymen who can be classified as centers of influence.

We at Beacon are confident that the roughage analysis service is a highly effective tool both in arousing the farmer's interest in using new feeding procedures and in obtaining information on his roughage supply that will help eliminate some guessing in the feeding program. It also lends a significant assist in stimulating the dairyman to take more interest in the proper feeding and handling of his cattle.

It is helpful to use data such as those found in Table 3, to focus attention on the financial advantage in handling each cow in the herd in a manner that will enable her to express

TABLE 3

High production and high feed costs are consistent with maximum returns[a]

	Average yearly milk fat production					
	304	351	398	445	496	541
Cost of roughage	$ 90	$ 95	$ 99	$104	$112	$109
Cost of grain	82	93	104	114	128	143
Return above feed cost	172	218	258	304	343	355

[a] New York D.H.I.C. Yearbook, 1957.

her inherited milk-producing capacity. Altogether too many dairymen today feel that the answer to the cost-price squeeze is to increase cow numbers and decrease per-cow feed costs. Rather, in our judgment, the data in Table 3 clearly indicate that dairymen who are not giving each cow every reasonable opportunity to perform according to genetic capability and are lowest feed cost oriented, probably are concurrently experiencing low returns over feed costs.

In summary, it is our judgment that the effective use of feed information on the farm can be implemented by the following procedures:

A. Use of larger numbers of higher-producing cows over longer experimental periods in dairy cattle research.

B. Translate dairy cattle research data into dollars and cents wherever possible.

C. Provide dairymen with specific feeding programs aimed at enabling the higher-potential cows to express their inherited production capability.

D. Innovate new methods to stimulate dairymen not testing to obtain production data in some manner on each cow, at least monthly. Ameliorate problem in some areas of shortage and turn-over of milk testers.

E. Place increased emphasis on encouraging dairymen to visit fellow-dairymen who are effectively using the improved feeding practice in question.

F. When contacting a dairyman relative to a new practice, make a specific appointment to assure thorough coverage of the reasons for and the mechanics and benefits of the improved method.

G. Ready availability of the services of a well-trained dairy specialist, be he an extension dairyman, county agricultural agent, or a representative of a feed manufacturer. Increasingly, the more progressive dairymen will seek the assistance of those men and groups who have demonstrated their ability to provide effective service in a given phase of the dairy enterprise.

OUR INDUSTRY TODAY

MODERN CONCEPTS OF DAIRY PLANT MANAGEMENT

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Management started as an art; it is becoming a science. It is a premium item, focused on youth. Early business history found management a one-man show—a man with wide experience from coming up through the ranks. Smaller businesses dominated; subsistence was often the goal. Management reward was for the art of making the right decision at the right time in a majority of the situations. If he made the right what decision, the how decision wasn't too important.

The Industrial Revolution fostered cost reduction. The manager with a cost-reducing patent rose to the fore. Names like Kraft, Borden, Swift, and Kellogg were imprinted on the pages of management history. But, industrial patents were not enough; economics then came forth as a prominent tool of management. This was made manifest in general accounting. The inventory of resources was formalized by the balance sheet; economic growth by the income statement; and operating procedures by the budget. A basis for science was appearing. Cost accounting refined this new science; break-even charts established quantity of output and plant size.

Operations planning, control, and improvement were then subjected to the scientific approach with the growth of so-called industrial management. The father was Frederick Taylor, whose principles of management still stand out today. Engineering thus gained entry into management. Taylor held that science could come to all phases of management including scientific selection and training of people, as well as scientific relations of workers to management and to each other. Engineering and economics tended to develop a management science. Yet, this was a piecemeal thing. Mathematics and statistics, along with high-speed calculators, are tending today to weld this piecemeal pattern into an over-all science of management. Such development parallels the scientific automation of production and distribution processes. Scientific management does not displace the manager. In fact, its fastest-growing aspect is the science of the manager himself—the so-called behavorial sciences such as psychology, sociology, and the study of the decision-making process itself. A major thrill of the future is its promise to integrate managers into management science.

With this background, my paper will hedge-hop four essential modern concepts of dairy plant management: (1) Creation of a favorable management environment; (2) orientation of the firm to its environment; (3) balancing of current operations against future plans; and (4) solution of problems involving a large bundle of interrelationships.

CREATION OF A FAVORABLE MANAGEMENT ENVIRONMENT

A favorable management environment needs several things. I will discuss two of special significance: The first is an informed group of people; the second, a logical organization of these people. Possibly as a surprise, then, I emphasize first the comptroller function, not top management.

The decision-making process today is a rather complicated process involving several components (Chart 1). The most typical management problem deals with alternative sources of raw materials convertible by alternative processes into alternative products at varying rates. Moreover, these alternatives are subject to various limited operating facilities and usually limited market outlets or supply sources. The alternative raw materials, products, and processes have varying costs or values, usually denoted by prices. A complicated set of relationships combines these many factors. Usually, the objective is to choose a set of alternatives which will maximize profits. A favorable management environment gives the people in the business a fair chance to obtain this objective.

Alternative Raw Materials, Products, and Processes	Operating Facilities
Specified Relationships	Marketing and Procurement Conditions
Prices or Returns	Profits

CHART 1. A management problem complex.

The inside area (Specified Relationships) in Chart 1 represents the basic information mandatory for use by an up-to-date manager. I conceive of these data as primarily operational and essentially devoid of economics. They are power requirements, machine speeds, product formulas, storage requirements, package size, and the myriad of other such data necessary to run a business. The outlying areas of Chart 1 tend to involve planning and control—essentially managerial items. These items tend to be stock in concept, often fixed over time and limiting in their nature. These involve deci-

sions of choice, especially in the long-run. Labor quantities, machine capacities, market limits, and many other factors make up this group. You need such information for two broad areas, planning and control. Management activities are divided among activities you are planning or programming for the future, and those you are trying to control, to see if you accomplished what you planned.

A favorable management environment needs organization. A hard and fast rule can not be laid down, but at least recognition of fundamental areas of management is necessary. One such classification of areas gives planning combinations of products and size of business, planning operations, controlling operations, improving operations and methods, and handling personnel. Organization is necessary to allow delegation. Top management today can not handle all the responsibility of a business—businesses are too big and the problems too complicated. Thus, good management delegates responsibility. Delegation is not easy. It means decentralization which, in turn, means that a manager must condition himself to delegate and he must take steps to see that the delegation is carried out.

To make delegation work, the manager must do certain things:

(1) Policies must be clearly stated. If a person is accountable for discharge of a responsibility under certain rules of the game, he is due an expressed statement of those rules.

(2) Jobs must be defined. If a person must make an accounting for the discharge of a responsibility, he is due a clear statement of that job or responsibility.

(3) Goals must be set. It is not enough to know what you are doing; good workers want to know why they are doing it. Everyone wants to know how he is doing. Goals make up this measuring stick.

(4) Ideas must be communicated. Informed people work things out. Communication must not be one-way. Delegation will foster ideas at the lower levels; these must reach the front office.

(5) Controls must be established. Even though an executive delegates authority to do a task, he does not rid himself of responsibility for getting it done. You can not blame him for assuring control; he can not delegate without control.

Incidentally, this organization for managing by delegation is a complicated machine and its lubricant is the information system mentioned above.

ORIENTATION OF THE FIRM TO ITS ENVIRONMENT

Management today shows an awakening to long-range planning. Much of this is probably faddish, but the spirit is encouraging. Management too often is provincial. Professional analysts have helped promote this. We economists have slighted the external economies of the firm. Most members of this Association are even more guilty, I am afraid. Long gone is the day that planning within the firm is enough within itself.

Let us look again at Chart 1. Alternative raw materials today are not a local market function. A management ignoring alternative raw material sources is asleep. Products selection must encompass a range of alternatives. This society is substitute-conscious. In some sense, nearly every product is a substitute today. Management that has ignored the effects of diet trends of next year's product line is asleep. The boyish lure of managers for machines may mean rather modern processes, but I seriously wonder if we will lag in automation as we did in industrialization.

Or consider operating facilities. One of my first professional questions was to ask why food plants remodel continuously. As yet, the answers have not satisfied me. Likewise, consider marketing and procurement. Demand analysis has been slighted woefully. Especially slighted are the interrelations between operations and demand. Witness the fact that the whole area of distribution accounting is relatively barren. Cross elasticity data between products is a guessing game. The fact that companies see services more than products often goes unnoticed. At least, management uses a similar policy for service items with an income elasticity of demand many times that of the product accompanying the service. Prices and profits open up the whole influence of consumer reaction. But let's speak of another—the overt effect of government on prices. The industry stands in confliet with the proper social control. Yet, social controls will continue to come. Will management speak up here in planning, or will the more common passive attitude penalize them for after-the-fact action?

The trends here are many. We will not explore many, but let's touch one. Rebirth of a national conscience seems to be in the air. Over the years public relations in general and personnel cooperation in industry have both come of age. A growing social impact seems promised in automation. A recent book by an Englishman, Mangus Pike, has made a good case that a social rebirth may be the result of automation. On the other hand, we have had real exposures of violations of our moral code. The TV programs, union rackets, monopoly violations, payola, and others swell the list. National resentment to these is encouraging and we may be able to do something about them. This acute problem has, in part, grown out of the buyer-beware concept permeating our business. Throughout many industries some things taken for granted today are not within traditional concepts of good business morality.

Low morality can be poor economics. Economic pressure is a sad purveyor of improved

morality, but it may be an important one. Prices are volatile (and sometimes unpredictable), but a very powerful determiner of relative equity positions in our society. Our current welfare concepts dictate that we recognize this. Nonprice competition has not undergone the incubation period of social analysis, especially in economics. Thus, the norms, mores, and moral codes are not well established. Until they are, the moral responsibility will rest primarily on individual businessmen. It will not only be the businessman's social responsibility but his economic responsibility, as well. A bad actor within an industry today can pull the total industry down upon its small number of members.

Another element of the firm's orientation that intrigues me is the engineer's advice to work on the big problems first. Too often in managing a business we worry about cents when we should be concerned with dollars. Or, maybe, it's minutes when it should be hours. Or worse yet, it's machinery valves, sales points, or bacteria when it should be people. We think of motion-and-time-study men as too often looking at the trees and missing the forest. Yet, it was these fellows who taught that the proper approach to labor saving will put the big things first. Their approach has usefulness beyond the study of work methods. When considering any work method change in a plant, sketch a work sheet like this:

The idea is to catalog possible changes, starting in the upper left corner. Consider first the possible changes in company size or merchandising practices. For instance, this might be a switch in emphasis from retail to wholesale. A change at this high level will probably dictate a change in product size, kind, or number; a switch toward wholesale might call for more paper than glass, or a new set of products to round out the product line. Changes here will cause changes in materials handling methods and plant processes. These changes, in turn, will call for consideration of plant design, equipment selection, and layout. Finally, these particular changes dictate the work methods. The cause and effect run from left to right and not vice versa. The point is, don't try to choose between two work methods, one of which emphasizes the left hand and the other the right hand, when the desirable change in equipment will eliminate use of both hands! Management needs such orientation.

BALANCING OF CURRENT OPERATIONS AGAINST FUTURE PLANS

The problem of stocks and flows is a troublesome one in economic theory. I find little solace that it gives the business manager even more problems than the academician. It is really a problem of comparing current stock (or investment) against expected flow (usually of some payoff). The capital investment problem illustrates the manager's dilemma.

Often the flow of return gives strong economic leverage. We know the concept of leverage in elementary physics. We have the same thing in economics. Let us consider labor saved by capital investment (Chart 3). With a wage rate of $1.50, one hour saved each day has enough economic leverage to effect an initial capital outlay of nearly $4,000. This merely illustrates a whole family of such modern management problems.

Another way to get at management's view of this planning-for-the-future problem, is a good orientation of relative cost proportions. For example, capital cost is small relative to many other dairy plant costs. Such orientation is best achieved by scanning the typical income statement or cost breakdown of a milk plant. Where does capital cost stand relative to other costs? By any reasonable classification, capital cost is relatively small. Let us consider simple cost breakdown in fluid operations (Table 1). Milk is the main outlay item (currently about one-half of our total cost and expenses) in dairy plant operations—a fact so easily over-

Possible changes in:				
Company size, and merchandising practices	Product size, kind, and number	Materials handling methods, and plant processes	Plant design equipment, selection, and layout	Work methods

CHART 2. Diagram illustrating effects of various management changes.

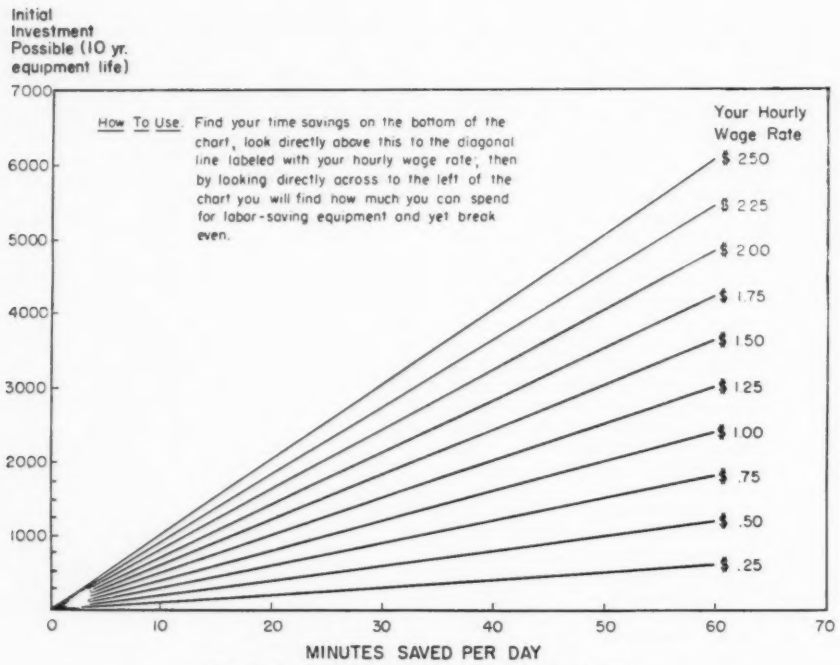

CHART 3. Labor savings vs. initial investment.

looked (Table 1). Other ingredients also add a per cent or so to the raw material. Labor is the next most important outlay (currently about one-fourth of our total cost and expenses). Containers take about 6 or 7% of the total outlay and advertising about 2%.

The basic charges normally associated with equipment are not large. Depreciation, repairs, rent, taxes, and insurance probably take about 9% of total outlays. Even if we add the other costs that go into our operation, we still have difficulty in finding more than about 1/6th of our total outlet in this area.

TABLE 1

Breakdown of costs—Average for clients of Edward B. McClain Company, Memphis, Tennessee, 1958

Item	Outlay per hundred-weight	Percentage of total costs and expenses
Raw milk	$4.99	51
Ingredients	.11	1
Labor	2.41	25
Containers	.63	6
Advertising	.16	2
Depreciation, repairs, rent, taxes, and insurance	.86	9
Other	.58	6
Total	$9.74	100

Management needs its deepest soul-searching here. Consider the worth of management time and capital investment again (Chart 4). The problem of current and future balance is emphasized by considering only some technological trends influencing mechanization for management. Note that I said for management, not of management; there is a difference. I want to develop this management and mechanization point, but please keep in mind that a similar problem exists for the problems management deals with throughout most of the operation.

Two imposing signs of our times backdrop today's management decisions in regard to mechanization—(1) the all-out attempt to make production processes automatic and (2) the companion attempt to routinize data-processing to assure the ultimate use of high-speed computing equipment in the management process. The first sign, automation, I will pass over this time; the second, I would like to comment on briefly.

Computers are under management scrutiny on three fronts. Production process automation often uses a computer as the focal point of control. Modern data-processing mandates con-

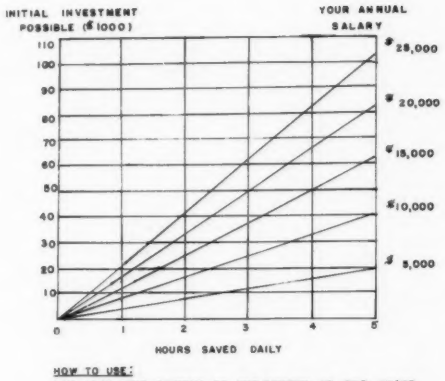
INITIAL INVESTMENT POSSIBLE ($1000)
YOUR ANNUAL SALARY
$25,000
$20,000
$15,000
$10,000
$5,000
HOURS SAVED DAILY
HOW TO USE:
FIND YOUR TIME SAVINGS ON THE BOTTOM OF THE CHART, LOOK DIRECTLY ABOVE THIS TO THE DIAGONAL LINE LABELED WITH YOUR ANNUAL SALARY; THEN BY LOOKING DIRECTLY ACROSS TO THE LEFT OF THE CHART YOU WILL FIND HOW MUCH YOU CAN SPEND FOR TIME-SAVING EQUIPMENT AND YET BREAK EVEN.

CHART 4. Management time saved vs. capital investment (10-yr. equipment life).

sideration of computers. Management problem solving increasingly makes use of computers. These three areas of computer application nearly encompass the sphere of management.

My comments often tend to be partisan toward computers, so I will yield to Ralph J. Cordiner of General Electric who said, "When the history of our age is written, I think it will record three profoundly technological developments: nuclear energy, which tremendously increases the amount of energy available to do the world's work; automation, which greatly increases man's ability to use tools; and, computers, which multiply man's ability to do mental work. Some of our engineers believe that of these three, the computer will bring the greatest benefit to man." Mr. Cordiner may or may not be right (1 and 2).[1] But our work in this area is extremely encouraging (3-6, 8, 9, and 13).

SOLUTION OF PROBLEMS INVOLVING A LARGE NUMBER OF INTERRELATIONSHIPS

Our technological treadmill of today creates two basic management needs for a well-balanced manager. First, up-to-date data he must have to make decisions is difficult to obtain and accumulate. I am speaking now about time standards, power requirements, machine capacities, costs, expenses, product formulas, advertising formats, and the myriad of other things provided by engineers, accountants, technologists, and salesmen. We mentioned them before.

Second, management needs techniques and systems with which to put these data together. This need arises because of the size of today's problems. The interrelationships among technical operating data are incomprehensible, irreconcilable, and sometimes apparently irrational to management. Paper-packaged milk takes more operating capital than glass-packaged milk. Yet, it stores better. Paper is a wholesale must, but glass may be preferred at retail; and retail customers are safe customers. And so on—you know the conflicting situations. How is a manager to know? He must have compromising and classifying techniques as modern as science can provide.

Let us assume you have the first management need—the technical ingredients. Let us further assume that you have learned to appreciate systems and techniques such as time study, cost accounts, break-even charts, budgets, and others. I would not slight these for the moment. They are powerful, underused, and under-rated. But I want to foster somewhat your similar appreciation for two newer techniques, linear programming and simulation. I choose these because they are tending to prove themselves in their own right. But, more important, they illustrate the two blades of modern quantitative management techniques—programming and applied statistics.

First, let us illustrate the use of linear programming on a typical problem of product line selection. This is a series of simple problems, but keep in mind that our continuing work expands these ideas as far as the economics of the problem leads them (13).

Problem I. First, let us take a very simple problem. Suppose:

1. We have these fixed items:
   (a) Cold-room space 10,000 sq. in.
   (b) Machine capacity 480 min. running time
2. It takes this much of each to produce one unit of these products:

	Cold-room space	Machine time
(a) Quart	30 sq. in.	2 min.
(b) Half gallon	50 sq. in.	5 min.

3. Our selling prices, less all costs except a charge for cold-room space and machine time, are:
   (a) Quart 9¢
   (b) Half-gallon 17¢
4. We can sell 1,000 quart equivalents of the two together at these prices. Either can substitute for the other.
5. We want to know which of these to produce to get the greatest return considering our fixed resources in cold-room space and machine time.

You look at this and obviously say, "Well, quarts net me 9¢ per quart equivalent and half-gallons only 8½¢ per quart equivalent, so I will push quarts. The market will take 1,000 qt. equivalents; have I machine time enough to produce this many? No, I can go up only to 240 qt. This looks like a bottleneck. Would I do better on half-gallons? No, I can produce

[1] Numbers of references cited.

only 96 half-gallons. This would return me only $16.32; 240 qt. would return me $21.60. So I had better stick to quarts. I know that 240 qt. are the most I can produce. But, maybe I can not produce even this many; have I cold-room space enough to handle them? A quick check shows I have enough to handle 333 qt.; so I am all right here. Thus, my answer—240 qt., no half-gallons."

You went at this much as the computer would and it wasn't too difficult. You didn't have many products, machines, or selling prices to deal with. It's when you multiply each of these several times that the computer comes into its own.

Problem II. Now, let's take a little harder one, and look at the machine's method a little more closely.

Suppose:

1. We have these fixed items:
   (a) Cold-room space 52,000 sq. in.
   (b) Quart machine time 360 min.
   (c) Half-gallon machine time 360 min.
2. It takes this much of each to produce one unit of these products:

	Cold-room space	Quart machine time	Half-gallon machine time
(a) Quart	8 sq. in.	0.04 min.	None
(b) Half-gallon	5 sq. in.	None	0.10 min.

3. Our selling prices, less all costs except a charge for the cold-room space and machine time, are:
   (a) Quart 25¢
   (b) Half-gallon 40¢
4. We can sell 10,000-qt. equivalents of the two together at these prices. Either can substitute for the other.
5. We want to know how much of each to produce to get the greatest return.

This is a little hard to see, so let us draw a picture of it (Chart 5). We know that we have capacity for only 3,600 half-gallons on the half-gallon machine, so let's draw a line in to show that we must not produce more than 3,600 half-gallons. This line is labelled "half-gallon machine capacity." The quart machine limits us to 9,000 qt., so we draw that one in as the "quart machine capacity" line. Now we know that the market will take either 10,000 qt. or 5,000 half-gallons. If we draw a line connecting these, it will show all possible combinations of the two which we could produce and still not go over the 10,000-qt. equivalent limit of the market. For example, one such combination would be 4,000 half-gallons and 2,000 qt. We call this the market possibility line. Now we have one other factor—cold-room space. We have space for 10,400 half-gallons or 6,500 qt. If we draw a line between these points we have all possible combinations which the cold-room can handle. One such combination would be 4,000 half-gallons and 4,000 qt. We call this the cold-room space line. Now,

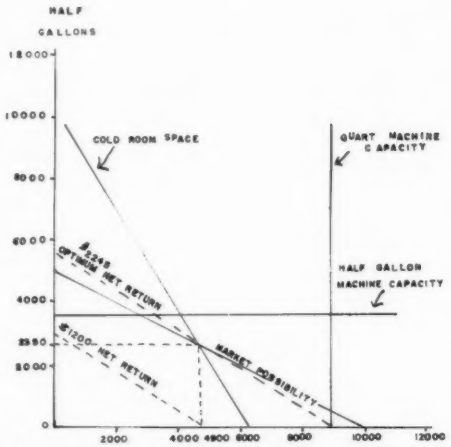

CHART 5. Illustration of linear programming solution for Problem II.

you can see that we have a picture of what we can produce—any combination of quarts and half-gallons below the heavy black line formed by these intersecting lines. The question is, which particular one? It's rather simple to find that one. All we need are the prices—factors we long ago found to be important in such a problem as this!

What are the relationships between our prices? All we need to determine this is to find how many of each we must sell to get a logical net return, say $1,200. We can get that with 4,800 qt. or 3,000 half-gallons. If we draw a line between these two points, we have all of the possible combinations of these products which will give us this return of $1,200. This line is labelled $1,200 Net Return. Now, if we will draw a line parallel to this one at the highest point possible, and yet touching the heavy line marking out our production possibilities, we can get our answer. Our line labelled optimum net return shows we should produce 2,550 half-gallons and 4,900 qt., for a net return of $2,245. Thus we produce at that level where this line touched our production possibility area. This is the most we can possibly get under our conditions. It is the one best answer.

The technique is a useful one for simple problems involving only two products, but when you get more than two, you have trouble drawing pictures of them and need another system. If you remember just a little of your algebra, you can visualize how the machine puts the above ideas into these mathematical equations:

Let: Q = Quarts produced
H = Half-gallons produced
M = Market not sold
C = Cold-room space not used
M_1 = Quart machine time not used
M_2 = Half-gallon machine time not used

Then

$$Q + 2H + M = 10{,}000 \text{ (quart equivalent of market)}$$
$$8Q + 5H + C = 52{,}000 \text{ (square inches of cold room space)}$$
$$.04Q + M_1 = 360 \text{ (minutes running time on quart machine)}$$
$$.10H + M_2 = 360 \text{ (minutes running time on half-gallon machine)}$$
$$\$.25Q + \$.40H = \text{Maximum dollars.}$$

Now, by solving these equations you get the same answer as on the chart. If you had only two products involved, you would probably use the chart. However, with equations such as these you can keep adding products and limiting factors and still make sense, even though you can not draw a graph of it. You probably visualize problems here in what products to produce, what raw materials to purchase, labor scheduling, work methods improvements, inventory control, what processes to use, best use of machines, and many others.

Two major expansions of this type of model have been developed in recent work. It is now possible to incorporate into the model price-making as well as price-taking concepts (12). Also, the recent work on integer solutions to linear programming allows us to make use of traditional cost accounting concepts of fixed and variable costs (11).

Illustration of the companion developments in simulation, using applied statistics in management computer applications, can be made with a recent planning problem of a cheese manufacturing plant (10). This problem again involved what and how much product to produce. The decision had to take into consideration: (1) availability of raw materials and their price structure; (2) plant capacity; (3) labor available and; (4) demand and price for the product manufactured.

Traditional analysis has not been able to incorporate explicitly into most models the stochastic characteristics of the external environmental factors playing on a management decision. In most analysis, especially in the so-called economic-engineering approaches, case studies, before-and-after illustrations, or replications, were necessary to test the synthesis under operating conditions. Attempts to develop shortcuts to gain the advantage of experience have been many, the least of which has not been formal education. In this type of problem the computer gives a promising approach most appealing to an economist.

In this study a simulation procedure was developed for analyzing dairy manufacturing problems under stochastic input conditions. The simulation procedure allows for the evaluation of various operating conditions without the need of actually implementing them. Milk arrivals from farmer producers were analyzed and a procedure for simulating them was developed. This procedure allows management to do its planning, taking into consideration the extreme situations that can occur as a result of the probabilistic nature of milk arrivals. This simulation of milk arrivals allows management to plan in advance the labor requirements for the cheese-room operation. It was found that three crew sizes, four men, seven men, and nine men, could effectively handle the cheese manufacturing under a wide variety of milk volume inputs.

Results of various surplus milk purchasing policies were also tested. It was found that the cost of manufacturing cheese using only farmer producer milk was 34.12¢ per pound. The price per pound increased to 34.69¢ when an additional 3,000-gal. tank truck of milk was purchased each day between October 1 and April 14. This surplus milk was purchased at a 50¢ premium over the price schedule paid to farmer producers. The price per pound of cheese was 33.77¢ when an additional 3,000-gal. tank truck of milk was purchased each day between April 15 and September 30. This milk was purchased at a 25¢ premium. The price of cheese was 34.32¢ when both purchasing policies mentioned above were carried out. Another purchasing policy involved purchasing all the surplus milk of a number of fluid plants. This policy introduced the addition of another unknown raw material supply. This milk was purchased at a premium of 25¢ per hundredweight. The cost of cheese under this policy was 33.70¢ per pound.

Simulation procedures also allowed the investigation of the efficiency of labor utilization under the various policies. It was found that the purchase of slightly higher quantities of surplus milk would give more efficient labor utilization.

Other applications of such concepts to agricultural firm management are impressive and growing in number (7). The future use of computers in this area is assured. Modifications of mathematical programming and applied statistical models tend to offer great promise and challenge. The crying need in this area today is for management-orientated people who are well enough acquainted with mathematics and statistics to be able to interpret this promise and challenge for management not equipped with mathematics and statistics. This gulf between the believers and the doers has been narrowing, but within this realm lies the future of computers in management.

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WHEN IS A QUART OF MILK A QUART?

B. L. HERRINGTON
Department of Dairy and Food Science, Cornell University, Ithaca, New York

Plants bottling milk buy milk by weight but sell it by volume. For accounting purposes, and for estimating plant losses, it is necessary to convert quarts of various products to pounds. We need accurate information concerning the weight of a quart of each product sold, but the weight of a quart depends upon the temperature at which it is measured. If a quart container is filled with cream at 70° F., it will be underfilled at 60°. If it is filled at 60°, it will be underfilled at 50°. If the seller measures the cream at 70°, has he fulfilled his obligation to deliver 1 qt. to the purchaser? Or should it be measured at 60, or 50, or 40° F.?

This is clearly a legal question, but in most states there is no legal answer. Letters were sent to the Chief of the Bureau of Weights and Measures in 50 states. Forty-eight replies were received, signed by a variety of officials, because in some states there is no Bureau of Weights and Measures. Presumably, the replies came from the official best-qualified to answer. Only California and Florida reported specific legislation on this point.[1] In these states, the law specifies that the measure shall be filled at 68° F. (20° C.). This conforms with the general regulation of the Federal Food Drug and Cosmetic Act: "A statement of liquid measure shall be in terms of the U. S. Gallon of 231 inches and quart, pint, and fluid ounce subdivisions thereof, and, except in case of frozen food which is so consumed, shall express the volume at 68° Fahrenheit (20° Centigrade)." Title 21, Part 1, Section 1.8 (h) (1958).

Many of the replies cited the requirement of the Health Department that milk be kept below 40° or 50° F. This is irrelevant to the question, for Health Departments do not have jurisdiction over weights and measures. It should be remembered that the temperature of measurement for proper fill is not necessarily the temperature of filling. In many states, it is customary to allow bottles of milk to warm to room temperature before examining them for fill.

It would be desirable for all states to have legislation concerning the temperature of measurement when liquid milk products are sold by volume. Such legislation is needed for the protection of both buyer and seller. To avoid confusion, the temperature should be the same in all states, and for all liquid dairy products.

The temperature of 68° F. (20° C.) seems well-chosen for several reasons. It is the standard temperature for the calibration of laboratory glassware and glass milk bottles. It is in reasonable conformity with the present practice of checking fill at room temperature. A lower temperature might not allow sufficient head space for the expansion of heavy cream when it is warmed. The legal fill-point is normally ¼-in. below the cap seat. This allows 5.0 ml. head space in a bottle with a neck di-

[1] In spite of replies to the contrary, A. J. Powers of the Borden Company has shown me evidence that the following additional states do have laws or official regulations specifying the temperature of measurement: Alabama, Arkansas, Illinois, Louisiana, Montana, North Dakota, and Washington. In each case, the temperature is 68° F.

ameter of 1.25 in. In practice, the head space is often less than 5 ml., because filling machines do not make perfect measurements and they must be so adjusted that the average fill is correct.

The amount of head space needed by different products when warmed to 75° F. depends upon the filling temperature. The following table shows the average expansion of samples purchased from five different dealers. The maximum value for cream may be appreciably greater.

Measuring temp. (° F.)	Head space required in quart bottles at 75° F.		
	Heavy cream	Milk	Skimmilk
	(ml.)		
40	17	5.1	3.5
50	13	3.7	2.8
68	2.8	1.1	0.9

It should be noted that adoption of 68° F., instead of a lower temperature, as the temperature of measurement, would have relatively little effect upon the quantity of milk or skimmilk in the bottles. The use of a lower measuring temperature could result in leakers in the case of cream. Leakers are unsightly and open the bottle to contamination.

The fill of paper containers is usually judged by weight, but we can not determine what the proper weight of any product should be until we first agree upon the temperature of measurement. This is a serious matter which deserves the attention of the industry. The difference between a quart of milk measured at 50 and one measured at 68° F. seems small, ¼%; but in terms of total sales, it represents a very large sum of money. There should be a satisfactory legal answer to the question: When is a quart of milk a quart?

ASSOCIATION AFFAIRS

CALL FOR PAPERS FOR THE 1962 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

M. E. Senger, Chairman, Program Committee, A.D.S.A.
Department of Animal Industry, North Carolina State College, Raleigh

The 57th Annual Meeting of the American Dairy Science Association will be held June 18-21 at the University of Maryland, College Park. Members who wish to present papers must submit titles and abstracts not later than March 1. This deadline must be met to permit publication of titles with the complete program in the April, and abstracts in the June, Journal.

All members of the Association, including graduate student affiliates, are entitled to present papers. Participation by members of the industry and by senior members of the Association is especially encouraged. The Program Committee favors the general policy that an individual present only one paper and that his name appear as author on no more than two. This Committee, together with the Association membership, wishes to stimulate vigorous, sound research and realizes that these restrictions may penalize some members engaged in full-time research. Therefore, the Committee has liberalized this policy so that a member may exceed these limits, but only if he or his department rates the abstracts in order of preference for oral presentation.

Papers submitted for the annual meeting should be confined to research that has not been reported. Abstracts of papers accepted for publication by a scientific journal before the annual meeting are not eligible, as this constitutes duplication. If the total number of papers submitted by the membership is too great to include in the program, the Committee will assign some papers to be read by title only. In this event, consideration will be given to quality of research to be reported, number of abstracts per author and department, and importance of the research. Abstracts arriving late will be rejected.

Attention is called to the Dairy Manufacturing Extension Section program and members are urged to participate. This is a subsection of the Manufacturing Section.

Mimeographed copies of pertinent data are desirable for distribution when the paper is presented. At least 250 copies should be made available. This can be supplemented by slides for projection on screens, provided the author can adhere to the assigned time of 12 to 14 minutes for presentation of each paper.

PREPARING ABSTRACTS

The Program Committee encourages continued improvement in quality of papers and in oral presentation. Careful design of experiments and proper interpretation of results are necessary. Strict compliance with the instructions for preparation of abstracts will simplify the task of the Program Committee, and will improve the program. *Careful editing of abstracts before submission is essential.* Each

year a number of abstracts must be returned due to incomplete details. Please follow instructions for preparing abstracts carefully.

1. All abstracts must be submitted on regular 8½- by 11-inch paper.
2. Abstracts must not exceed 200 words by actual count. Those exceeding 200 words will be returned to the author for revision.
3. An original (on bond paper) and three copies of each abstract should be typed double-spaced. The original and one copy should be mailed to the Chairman, another copy to the Vice-Chairman, and the fourth to the Secretary of the Section where the paper will be presented. The original copy will be used for publication in the JOURNAL.
4. The style and abbreviations of the JOURNAL OF DAIRY SCIENCE must be used. Please refer to abstracts in the June, 1961, JOURNAL for guidance.
5. Only initials of authors should be used, except in unusual cases where it may be necessary to use the complete name.
6. When more than one author is listed, indicate who will present the paper by an asterisk after his name.
7. The title should indicate clearly the nature of the research. It should not be repeated again in the text. The abstract should include, insofar as possible, the design and major results of the investigation. Only complete research should be reported. Brief, essential statistics will make the data more meaningful.
8. The following form with no caps for the title is correct: Utilization of carbohydrates posterior to the rumen-reticulum of the bovine. J. T. Huber and N. L. Jacobson, Iowa State University.
9. If the author lists an address for an experiment station other than the University, such as a USDA research branch or a commercial company, the complete address should be provided, as in the following example: A study of dye reduction methods as platform tests for the detection of antibiotics. Burdet Heinemann, Producers Creamery Co., Springfield, Missouri.
10. All symposium papers should be typed double-spaced and organized according to the style used in the JOURNAL OF DAIRY SCIENCE. The author should send the first copy direct to the JOURNAL Editor, E. O. Herreid, before or immediately following the Annual Meeting.

GRADUATE STUDENT PRESENTATION CONTEST

This contest will be conducted in both the Production and Manufacturing Sections. Each institution is entitled to enter one participant in each contest. They must be student affiliate members. Complete rules for the contest are being sent to department heads. Those wishing to enter the contest must submit copies of their abstract to the Section Officers as outlined above. They should also include a letter indicating their desire to enter the contest. This letter should also be signed by the major Professor and the Department Head. A carbon copy of the letter and four additional copies of the abstract should be mailed to the contest representative.

For Production Section Award: Louis Boyd, Dairy Department, University of Tennessee, Knoxville

For Manufacturing Section Award: A. V. Moore, Department of Dairy Science, Texas A&M College, College Station

Names and addresses of officers of sections to whom titles and abstracts should be sent are:

EXTENSION SECTION

Chairman: W. R. Van Sant, Department of Dairy Science, University of Arizona, Tucson.
Vice-Chairman: C. D. McGrew, Department of Dairy Science, Ohio State University, Columbus 10.
Secretary: D. E. Voelker, Department of Animal Husbandry, Iowa State University, Ames.

PRODUCTION SECTION

Chairman: J. C. Thompson, Ralston Purina Co., St. Louis, Missouri.
Vice-Chairman: L. H. Schultz, Department of Dairy Husbandry, University of Wisconsin, Madison.
Secretary: V. R. Smith, Department of Dairy Science, University of Arizona, Tucson.

MANUFACTURING SECTION

Chairman: M. L. Speck, Department of Food Science and Processing, North Carolina State College, Raleigh.
Vice-Chairman: D. M. Graham, Pet Milk Co., Research and Development Center, Greenville, Illinois.
Secretary: E. L. Thomas, Department of Dairy Industries, University of Minnesota, St. Paul.

(The Dairy Manufacturing Extension Section is a subsection of the Manufacturing Section. W. S. Arbuckle, Department of Dairy Husbandry, University of Maryland, College Park, is chairman, and A. L. Rippen, Department of Food Science, Michigan State University, East Lansing, is secretary. All abstracts for papers in this subsection should be submitted through the regular channels of the Manufacturing Section, but should be identified for presentation at the Dairy Manufacturing Extension Section meeting and a courtesy copy sent to W. S. Arbuckle.)

INVITATION FOR NOMINATIONS FOR VICE-PRESIDENT AND DIRECTORS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

A. C. Fay
Chairman, Nominating Committee,
12458 North Bay Shore Drive,
North Miami, Florida

Every member of the American Dairy Science Association is urgently requested to participate in the selection of officers and directors for the year 1962-63. In accordance with the By-Laws and established custom, and in order to maintain a balanced representation from the Sections, we are to elect this year:

(1) A Vice-President from the field of Dairy Manufacturing, who will automatically become President in the year 1963-64.

(2) One Director from the field of Dairy Production to serve for three years, and

(3) One Director from the field of Dairy Manufacturing to serve for three years.

As a member of A.D.S.A., you are specifically requested to submit your suggestions for each of these three important offices to the Chairman of the Nominating Committee before January 1, 1962, but preferably NOW, while it is fresh in your mind. In making these suggestions, consideration should be given to persons not only having inherent qualities of leadership but having demonstrated their devotion and interest in the affairs of the Association by serving as Sectional Officers or through membership on important committees. Experience in the business affairs of the Association is particularly important in your choice for the office of Vice-President.

On January 1, 1962, the Chairman of the Nominating Committee will assemble the suggestions received and request from some one of the nominators of each candidate a biographical sketch of his nominee for consideration of the members of the Committee. After selecting a slate of two candidates for each of the three offices and obtaining their acceptances, the list will be published in the 1962 April issue of the Journal of Dairy Science, and later ballots will be submitted to the membership by the Secretary-Treasurer of the Association.

Members of the Nominating Committee are as follows:

Ralph E. Hodgson, USDA, Beltsville, Maryland

Marvin I. Speck, North Carolina State College, Raleigh, N.C.

J. C. Thompson, Ralston Purina Company, St. Louis, Missouri

W. R. VanSant, University of Arizona, Tucson, Arizona

A. C. Fay, 12458 North Bay Shore Drive, North Miami, Florida, Chairman.

Please give serious consideration to this request for your cooperation and act NOW by sending your suggestions to Chairman A. C. Fay.

PRICE SCHEDULE FOR REPRINTS OF PAPERS THAT APPEAR IN THE JOURNAL OF DAIRY SCIENCE

H. F. JUDKINS, Secretary-Treasurer
32 Ridgeway Circle, White Plains, New York

The Executive Board, at the time of the Annual Meeting of the American Dairy Science Association at the University of Wisconsin, increased the price of reprints 25%, effective July 1, 1961. The new reprint schedule follows:

No. of reprints	Number of pages								
	2	4	8	12	16	20	24	28	32
	(Cost in dollars)								
50	17.50	20.00	36.25	51.25	67.50	78.75	97.50	115.00	125.00
100	20.00	22.50	41.25	61.25	77.50	92.50	112.50	132.50	145.00
200	22.50	28.75	51.25	76.25	97.50	117.50	143.75	162.75	185.00
300	28.00	33.75	62.50	91.25	117.50	143.75	173.75	205.00	226.25
400	30.00	40.00	72.50	107.50	137.50	170.00	205.00	241.25	266.25
500	33.75	45.00	83.75	122.50	157.50	195.00	236.25	277.25	306.25
600	37.50	51.25	93.75	137.50	177.50	221.25	266.25	313.75	346.25
700	41.25	56.25	105.00	153.75	197.50	246.25	297.50	350.00	387.50
800	45.00	62.50	115.00	168.75	218.75	272.50	328.75	386.25	427.50
900	48.75	67.50	126.25	185.00	238.75	298.75	358.75	422.50	467.50
1,000	57.25	73.75	136.25	200.00	258.75	323.75	390.00	458.75	507.50

If covers for reprints are desired, the cost of 50 covers will be $21.18, and for each additional 100 covers, the cost will be $8.75. Back copies of the JOURNAL will cost $2 each.

The reprints are made from standing type within 30 days after the papers appear in the JOURNAL. Requests for a few reprints of a paper should be sent to the authors, whose names and addresses appear with the title. The Secretary and the Editor's office do not keep supplies of the various reprints. Orders for large numbers of reprints should be sent to The Garrard Press, 510 North Hickory Street, Champaign, Illinois. These orders must be received within 30 days after the papers are published in the JOURNAL; otherwise, the type will have been destroyed.

In case the original type has been destroyed, it is possible to supply reprints by a special photographic process, and their cost will be 50% more than the regular ones. For example, 100 reprints of 32 pages will cost $217.50.

It is hoped that the publication of this reprint schedule will make it easier for interested people to obtain reprints in any number desired and, at the same time, aid in disseminating useful information to the dairy and related industries.

The JOURNAL OF DAIRY SCIENCE is copyrighted. Reproduction of complete papers from this JOURNAL by any organization is not permitted. The reproduction of graphs, tables, and illustrations for books and other periodicals may be authorized by the Editor-in-Chief.

BOOK REVIEW

LABORATORY MANUAL FOR DAIRY MICROBIOLOGY. E. M. Foster and W. C. Frazier. Burgess Publishing Co., Minneapolis, Minn. 72 pp. 1961. $3.

This is the third edition of a well-known laboratory manual. It consists of 30 experiments designed for 2-hr. laboratory periods and these are divided almost equally between the three-part headings: (1) study of milk microorganisms, (2) methods used in the control of milk quality, and (3) microbiology of dairy products. In general, this new edition contains substantially the same experiments, questions, and references as the previous editions. The manual has been brought up-to-date with respect to bacterial nomenclature and newly recommended laboratory procedures. Current interest in inhibitory substances in milk has been satisfied by the addition of a new experiment, and a few other improving changes have been made in the experiments. This new edition is an excellent laboratory manual for a beginning course in dairy microbiology.

LLOYD D. WITTER
University of Illinois, Urbana

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Direct assay method of Arret and Kirshbaum (FDA) for determining presence of penicillin in milk and dairy products.

Media	BACTO - ANTIBIOTIC MEDIUM I BACTO - SPORULATING AGAR
Inoculum	BACILLUS SUBTILIS ATCC 6633 Standarized Spore Suspension in 1 ml. ampuls
Penicillinase	BACTO - PENASE CONCENTRATE in 20 ml. and 100 ml. vials BACTO - PENASE DISKS Standardized Impregnated Disks
Penicillin	STANDARDIZED IMPREGNATED DISKS 0.05 unit, 0.1 unit and other concentrations

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